Exhibit A

IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF MARYLAND

SALLY W. TARQUINIO,	:	
Plaintiff,	:	
vs.	:	Case No.
JOHNS HOPKINS UNIVERSITY	:	1:23-CV-00727-RDB
APPLIED PHYSICS LAB,	:	
Defendant.	:	

Videotaped Deposition of SALLY TARQUINIO

Greenbelt, Maryland

Monday, October 16, 2023

9:33 a.m.

Job No.: 510781

Pages: 1 - 209

Recorded By: Natnael Assefa

C O N T E N T S

E X H I B I T S

(Attached to transcript.)

E X H I B I T S (Continued)

(Attached to transcript.)

limited circumstances is the expectation that you don't -- is the -- the -- except under very limited circumstances, the expectation is that you answer all the questions that I ask.

I will try to stick with things that are very relevant to the claims. I will stick with things that are about your own allegations or the defenses that are at issue in this case. I will not ask you questions about, say for example, conversations you've had with your lawyer because you understand that those would be privileged; right? Correct?

A Correct.

Q Okay. But your -- your lawyer may object to one question or another, but after that objection, unless he instructs you not to answer, you can go ahead and answer, okay?

A Yes.

Q All right. Let me ask you this, when did you first start working at the Applied Physics Lab?

A I believe it was 2005.

A And there are people.

Q There were accommodations that were afforded to some folks; correct?

A Correct.

Q Okay. Absent in accommodation, the policy applied to everyone at the lab; correct?

A Correct.

Q Okay. And let me ask you this --

A Depending on how vocal -- you had an advocate that -- that took your case up with management.

Q All right. Again, the policy as announced applied to everyone at the lab; correct?

A Correct.

Q Okay.

A And it was applied differently depending on what part of the lab you were at; how strong your group supervisor was; how strong your various parties were.

Q Okay. What evidence do you have to support that allegation?

A I know that when I had asked to have my

group sup to -- to have my program manager support, he -- he was told that he could not do that. It had to do with the group supervisor who had just been promoted. So that is what I personally experienced.

Q Well, you didn't personally experience someone telling the group supervisor that they couldn't be involved in the process; correct? That's just what you were told; is that right?

A She said that she wasn't -- no one contacted her, she was in the dark. No -- no one had asked her certain things.

Q You dealt with a number of people who were in charge of processing the accommodation requests; correct?

A The only person that I actually had a conversation with, I -- I forget her name, but she basically didn't appear to understand how the lab is set up and that I had been working there the entire time. So she's -- she was asking questions such as do -- is there six-foot distancing? I've been doing six-foot distancing the entire time.

Q All right. Many people at the lab -- so let me ask you this, pre-COVID, the lab didn't institute distancing policies; correct?

A Correct.

Q Okay. And during the pandemic leading up to the enactment of this policy, the lab did enact certain policies like masking, or distancing, or testing; correct?

A Correct.

Q Okay. And when the lab announced this policy of mandatory vaccination, that was a change from what had been going on previously; correct?

A Correct.

Q Okay. And that policy applied again, absent accommodation, that policy applied to every lab employee; correct?

A Correct, and I follow those policies, I was happy to do so.

Q I'm asking about the -- the vaccination policy. Up until the vaccination policy was enacted or the vaccination policy applied to everyone at the lab absent accommodation; correct?

A As I understand it.

Q Okay. And that policy was a change for everyone at the lab? The policy of the mandatory vaccination was a change for everyone at the lab; correct?

A As I understand it.

Q And there were many people like you who were working physically in person, who were distancing, who were testing for some period of time; correct?

A Yes.

Q Okay. But that -- but when the policy was enacted or announced, that was a change in policy for all those people, not just you, for all those people who had been doing those things that you're mentioning; correct?

A As -- as was told to people.

Q So that's a yes; correct?

A Yes, that's what we were told.

Q Okay. But that's actually what happened. Some people got vaccinated, some people did not, some people were accommodated, and some

people were not, as far as you understand it; correct?

A Correct.

Q Okay. During your time at the lab, there were many workplace policies that applied to all employees; is that right?

A Yes.

Q You're probably aware of the anti-discrimination or harassment policy, something like that; correct?

A Yes.

Q Okay. There's an ethics policy and there's an accommodation policy, there's all kinds of different workplace policies that all employees are expected to adhere to; correct?

A Correct.

Q Okay. And so those policies are a job requirement for everyone; is that right?

A Correct.

Q Okay. And when issues arise about those policies and whether or not they've been violated or whether or not they apply to any individual

employee, questions about those policies, they would be job-related questions; right? Because it's a requirement for everyone in their job; is that right?

A Yes.

Q Okay.

A Including HIPPA.

Q Let's go to Paragraph 7 of the complaint. It says here you were -- or in 2004 and again in 2012, you were diagnosed with and treated for a Lyme disease. Do you see that?

A Yes.

Q Okay. Why don't you tell me a little bit about how you came to be diagnosed in 2003 or 2004 with Lyme disease?

MR. SCHIFANELLI: Objection to the extent that he calls for a medical expert.

BY MR. SCHNEIDER:

Q Sure. But what is your understanding of your diagnosis?

A I -- my doctor had used his diagnostic tools and whatnot and I was treated for about a

involved in the decision to terminate your employment?

A No.

Q Okay. You no longer work with that person at the time that you were terminated; correct?

A Correct, but I -- he was an example of what I knew. It's not something that you -- you basically -- what counts as your performance, not whether what you're dealing with. So people have cancer and -- and if that's what they want to share, that's fine. But I dealt with what I did and it did not affect my performance.

Q Okay. But it's not your position in this case that there was some deficiency in your performance; correct? That the -- the lab didn't -- the lab didn't terminate you, for example, because you had some performance issue?

A Correct, in fact, I had just been promoted.

Q Correct, and really what the lab ultimately did was terminate you because you

weren't in compliance with this policy; is that right?

A Correct.

Q Okay.

Q In 2012 -- well, let me ask you this. In 2003 or 2004, what are the names of the doctors that were treating you at that time?

A Ron Murray and Dr. Ross.

Q Ross?

A Right, I can't remember his name. He's since deceased.

Q Okay. And then you said in 2012 you found Dr. --

A Sivieri.

Q Sivieri. Okay. And how did you find him?

A I had heard from friends that he was very good and what I did is I knew that -- well, what actually happened was I had received a bite and it created this crazy thing on my skin. And I Googled it and it said, whatever you do, go to a doctor.

And I believe that the line from the first case, whether it was from a mosquito and this was from a mosquito, and so I kept calling, you know, usually you have to wait like a year to see Dr. Sivieri, usually a year and a half for Dr. Schwartz at the time, but who I didn't know at the time.

And they -- because I kept calling every other day because I knew that I had a limited window in terms of treatment of, you know, the more you wait, the more it takes hold. And then from there, I just explained what my symptoms were, what -- what I was experiencing, that's all I did, was tell him that.

Q Okay. From 2012 until before you requested an accommodation from the vaccine policy, did you tell anyone at the lab that you had Lyme disease or that you suffered from long Lyme or immune dysregulation?

A No, because again, my experience from hearing from other people and from what I heard observed from -- from that one group supervisor is

that would have been detrimental to my career --

Q Okay.

A -- because of their false belief of what it entailed.

Q Okay. And so -- but again, that group supervisor was not someone you worked with at the time you were terminated; correct?

A Peripherally, I did still work with him.

Q Okay. As far as you know, they weren't involved in this decision to let you go; correct?

A Correct.

Q Okay. And the people who were involved they --

A There are other people that again -- go ahead.

Q Well, I was -- okay. We can move on.

A Let's go to the next page, Paragraph 11. There's -- the paragraph here says, at all relevant times, although Plaintiff suffered from the effects of long Lyme, she was capable of filling the essential functions of her position. Let me ask you a little bit about your job at the

A Correct.

Q And they did so in compliance with the policy; correct?

A That's -- I can't speak to them.

Q Okay. Let's go to number 14. You say here in this first part, Although Plaintiff at the time of this requirement had no active lyme pathogen in her bloodstream. Do you see that?

A Yes.

Q Okay. And so that's true? You -- you did not at this time have any active pathogen for lyme in your bloodstream?

A I had no blood tests that showed that I had an active lyme pathogen.

Q You did later, though; is that right?

MR. SCHIFANELLI: An objection. Calls for medical expert testimony.

THE WITNESS: Yeah.

MR. SCHIFANELLI: Then you can answer.

BY MR. SCHNEIDER:

Q Go ahead.

A As I understand it, Lyme never goes

away. It's always there.

Q Okay. But as far as a blood test goes, I mean, even if you -- you allege it right here that at that time you had no active Lyme pathogen in your bloodstream. Are you saying that that statement is not true because you have this understanding that Lyme doesn't go away or is this actually true?

A It's true. Basically, I -- I don't have symptoms. I didn't have any issues.

Q This doesn't talk about symptoms though. This talks about pathogen in your bloodstream. So is it true that at this time you did not have an active lyme pathogen in your bloodstream?

MR. SCHIFANELLI: And again, objection.

THE WITNESS: I -- I --

MR. SCHIFANELLI: Let's make it a continuing objection.

THE WITNESS: I don't -- this would be up to my doctors.

BY MR. SCHNEIDER:

Q But you wrote it here. So is it not

effects associated with chronic Lyme. So at the time that the lab's policy was announced, how were you continuing to suffer from the effects associated with lyme?

A Essentially, I needed to follow my maintenance protocol just to make sure everything stayed same.

Q Okay. So at that time, were you -- were you actually suffering any ill effects or were you --

A No.

Q -- just maintaining?

A Maintaining.

Q Okay. And so I guess it would be accurate to say then at that time, you were having no issues doing your work or, you know, taking care of yourself or things like that?

A Correct.

Q Okay. So you were able to function relatively normal like your -- your major life activities, you were good to go?

A Correct.

MR. SCHNEIDER: -- to her?

(EXHIBIT 2 MARKED)

BY MR. SCHNEIDER:

Q Okay. Do you recognize these --

A Yes.

Q -- documents? Okay. Let's go to the first page. Do you recognize this e-mail?

A Yes.

Q Okay. And so this is an e-mail that you sent to the lab's accommodation coordinator on October 1st?

A Correct.

Q Okay. And this is the e-mail that you sent in response to the announcement of the vaccine policy to --

A Correct.

Q -- request an accommodation; correct?

A Yes.

Q Okay. Let's go to the second page. This document is called, at the top here, Employee requests for Medical Accommodation. Do you see that?

the patient.

Q Okay. Let's go to the top part. There's a paragraph here that begins, You may request. Do you see that?

A Yes.

Q Okay. In the middle of that paragraph, it says, In the event that the lab needs to obtain from you and/or your physician information regarding the condition for which you are seeking reasonable accommodation, you will be asked to supply such information and/or asked to provide a written authorization for the lab's medical office to contact your physician. Do you see that?

A Yes.

Q Okay. And so at the time that you signed this, you understood that the lab may come back and ask you some questions about the forms that you were submitting?

A I thought they would -- they could ask, yeah.

Q Okay. That's all I'm asking. You understood that they could or that they may;

correct?

A Yes.

Q Okay. And you also understood that they may ask you to authorize the lab's medical office to contact your physician; correct?

A According to this, but I was so focused on the fact that, you know, that I was being put in a rock and a hard place.

Q Okay.

A And I wanted to know whether to follow my doctor's advice or not.

Q Well, let me ask you this --

A So my doctor and I had been in contact before this date, but --

Q When had your doctor and you been in contact before this date?

A When I had asked to set up an appointment, and this is the date that he was able to give me.

Q Okay. And so you called and talked to Dr. Schwartz about your appointment ahead of time or you talked to his office to set the

appointment?

A I -- I thought I talked to him, but --

Q Okay.

A -- I don't recall.

Q All right. And that was in the two-week period or the month-and-a-half long period between when the policy was announced and when you had this appointment? All right. Okay.

Okay. Let's go down to number one. You write here, What accommodation are you requesting? And you wrote, Exemption from the COVID vaccine requirement and COVID-19 testing. So I want to understand -- I want to understand what you mean here. You're asking for an exemption from the vaccine requirement?

A Correct.

Q And then are you also asking for an exemption from the COVID testing?

A At the time, I did, yes.

Q Okay. And why were you asking also for an exemption from the COVID testing?

A Because I didn't think it was

reasonable.

Q Okay. But you testified earlier that --

A Right, and you'll see other e-mails where I just -- where I said, okay, I'll continue it. I mean, it was very --

Q No, I understand. I'm -- I'm trying to understand what you were -- what you were requesting at this time. And -- and why? So you testified earlier that the issue with regard to your condition for not taking the COVID vaccine is you don't want to introduce a foreign --

A Correct.

Q -- antibody into your body. Why -- why is the testing related to that?

A Testing of viruses is elusive.

Q Let me ask you this. So is -- is it -- is it correct that you just had kind of a generalized objection to subjecting yourself to testing? It didn't have anything to do with your medical condition; correct?

A Not unless the testing involved putting an antigen in me, I mean.

A I guess.

Q Okay. So why did you ask for it? That's what -- I'm trying to understand why. Just because you didn't want to do it?

A I -- I don't believe that it actually helped the situation. Again, the entire time that I was there, I was not sick, but the people that were sick and took tests were -- were sick.

So to me, the thing that would help is to address any, you know, help your immune system, help with viruses. To me, the testing doesn't do anything. But I -- I realized that really, if that's what it needed for people to feel safe, I -- I mean, I did it.

Q Okay. So again, I think I'm just trying to understand why. Is it your testimony that you just didn't think that the testing would help, so you asked to not be able to do it?

A I really -- I hadn't thought about this for quite a while. So I'm --

Q Well, let me ask you this.

A -- I'm trying to remember what it was --

understanding.

Q No, I am. I'm asking you specifically about your symptoms they -- they change

A I'm -- I'm saying that as levels of toxicity is added that -- it's like you're giving fuel to the --

Q But none of your doctors --

A -- to the line.

Q -- provided information to the lab directly that said that; correct?

A As if it would make a difference.

Q Yes, it would. Because you testified earlier, you're not a doctor; correct?

A Correct.

Q And although you may know a lot about your own journey with Lyme disease and immune dysregulation, you're not a medical expert, you're not a provider.

A Correct.

Q And then you're not qualified to decide just whether or not you're able to take one vaccination or another or take whatever.

A Yeah.

Q That's an opinion that you would work off of from your medical providers; correct?

A Correct.

Q Okay. And the information that you provided came from you largely and then also was nine years old; correct?

A Yes.

Q Okay. Let's go to the second page here. Do you recognize this document?

A Yes.

Q Okay. Am I correct that other than where Dr. Schwartz signed and when he dated and put his phone number, and his -- I guess, maybe his health care provider license number that you filled out the rest of this?

A Correct.

Q You checked the box here on other?

A I asked him -- I don't remember if I actually did the box or not.

Q Well, you wrote here chronic --

A But I wrote that --

Q Right, but this is the employee form. What you're looking at is the employee form; correct? It says employee requests for medical accommodation on it; correct?

A Right.

Q Okay. And then this form is the medical verification, the provider form; correct?

A Yes, but I --

Q And that's why you had Dr. Schwartz sign it --

A Correct.

Q -- because he's the provider.

A And he -- he saw this too.

Q I get that. I'm not disputing that. I'm saying it says right here that you needed to provide -- the doctor needed to provide information in a separate narrative that describes the reason in detail.

And so is it your testimony here today that this three or four -- three words and plus five words is a detailed narrative that describes from your provider what -- not only what your condition

A Yes, I would be surprised.

Q Okay. We're going to go through those e-mails, and I'll represent to you right now that you don't say that in any of them. This is the employee form, and you submitted it. And this is the provider form that you submitted. This right here under other only discusses from -- only discusses what your condition is; correct? Chronic Lyme disease and Lyme-induced immune dysregulation; is that correct?

A That's the condition.

Q Okay. And I'm going to come back to the -- kind of the thing we started with is that is there any information provided directly from your provider to the lab at any point that explains why because of your condition, you cannot be -- that you -- that you need to be accommodated from this policy?

A He provided those two journal articles.

Q Right, but those journal articles are -- are almost 20 years old; aren't they? One of them is from 2004; is that correct?

A The -- the body works the way the body works.

Q Right, but those studies weren't about you; right?

A It's about -- it's about the immune dysregulation.

Q I understand that. But let's go back to the -- the narrative, flip one page. Let's go back to other. Seeing the parentheses there, can you read what it says there?

A These requests were reviewed on a case-by-case basis.

Q Correct me if I'm wrong. I'd imagine that you would want your requests for accommodation to be considered as you as an individual; right? With your specific medical history, your specific health reason for not being able to receive the vaccine. I expect that that's what you would want.

A Yes.

Q Like, you are different than other people who have Lyme; right? There are some

people who had Lyme disease who had it pretty easy, like my wife, for example, a couple of rounds of antibiotics and she was good to go. And there are some people who may have suffered from Lyme that has had it worse than you; right?

A Correct.

Q And then there are people who have the same condition, the other condition that you have, a Lyme-induced immune dysregulation. And there are some people who have a harder time and some people have an easier time than you; right? You would just imagine that that's the case by odds.

A (No verbal response.)

Q Can you say yes? I know you're -- you're nodding.

A Yes.

Q Okay.

MR. SCHIFANELLI: I'm going to object. It sounds like you're testifying.

THE WITNESS: It does sound like that.

BY MR. SCHNEIDER:

Q Well, I'm just asking. Correct, you

didn't apply to me.

Q Then why not -- why -- no, never mind. Let's strike that.

Let's go to Exhibit 3. Do you recognize this document?

(EXHIBIT 3 MARKED)

A Yes.

BY MR. SCHNEIDER:

Q Okay. This is the lab work that we were just discussing from 2012 that you submitted in support of your accommodation requests; correct?

A Correct.

Q Okay. I know you're -- you're nodding, I just go with yes. Okay. This is -- this lab work was ordered by Dr. Sivieri as it indicates here at the top; correct?

A I was not able to get an appointment with Dr. Sivieri, and given the time -- limited time that I had available, that's why I had to go with the nurse practitioner.

Q Okay. I think we're conflating two times. This is the 2012 blood work?

A That's Sivieri, I only talked to him.
Q Okay. And so --
A I did meet the nurse practitioner and she did say completely different things than he did. So I -- yeah, apologies.
Q Okay. The date on this is June 30, 2012; is that right?
A Whatever's on the form is correct.
Q Okay. So yes?
A Correct, yes.
Q Okay. And that's it. Let's go to Exhibit 4. Do you recognize the e-mail?
(EXHIBIT 4 MARKED)
A I hadn't remembered it, but yes.
BY MR. SCHNEIDER:
Q Okay. At the bottom here is the e-mail that we looked at earlier; right? Where you submitted your requests for accommodation. And the date for that e-mail is October 1; correct?
A Yes.
Q Okay. And above this e-mail is from a woman named Elizabeth Bunda-Lee. Do you see that?

modified, but that's what I was doing at the time.

Q Okay. Let's go back to the complaint, Let's look at Paragraph 15. It says here, after acknowledging receipt of Plaintiff's accommodation requests and requests for exemption, which is the e-mail we looked at before. APL employees who were handling the matter asked Plaintiff to submit another APL form which was called Requests for Personal Medical Records, do you see that?

A Yes.

Q Okay. And you recall that that's accurate, they did ask you to send -- to sign that form; correct?

A Well, you showed that, you do have to realize too that, yes.

Q Let's go to the end of the -- the same paragraph on the next page. There's the last sentence there. It says, Plaintiff declined to sign the medical -- the requested medical release, is that -- that's an accurate statement?

A I didn't sign one.

Q Okay. And so you declined to sign it;

correct?

A Yes.

Q Okay. And you right here in the sentence above that, the same employees notified Plaintiff that the form would enable APL's medical officer can consult with your medical provider on any necessary follow-up. So, you understood that that was what the Lab was asking you to sign the document for; correct?

A My -- again, my understanding is that you wanted to have all my detailed medical records and I had an issue with that because to me that's private information. And so that's what I was responding to and I was focusing on doing my job, I was trying to -- we had a number of tasking going on.

Q Okay. But you knew that, and you write it right here, that the same employees notified you that it would enable APL's medical officer to consult with your -- with your Doctor; correct?

A Yes.

Q Okay. And so and you declined to allow

them to speak with your medical provider, among whatever else the form would have also requested?

A Yes.

Q Okay. Let's go to Paragraph 16. It says here, no APL employee reached out to her to query how APL may reasonably and safely accommodate her employment issues since her disability, as explicitly stated by her treated physician, precluded her from receiving the experimental mRNA vaccines, do you see that?

A Yes.

Q Okay. Where did your Doctor say explicitly, that you were precluded from receiving the experimental mRNA vaccines, what information did you provide from your provider where they explicitly said you were precluded from receiving the experimental mRNA vaccines, what document did you provide the lab that said that?

A That was just an understood. It was experimental, and it was mRNA which impacts --

Q Okay. We can get to the mRNA part. I'm asking you as explicitly stated by her treating

physician, precluded her from receiving the experimental mRNA vaccine, what document or piece of information from your provider, did your provider explicitly say that you were precluded from receiving those vaccines, which document?

A -- any antigen, he focused on any antigen.

Q All right. But -- but you're asking about you said it right here in an amended complaint which you testified earlier, has to be truthful and honest to the best of your ability. You said that your Doctor explicitly stated that you were precluded from receiving the experimental mRNA vaccines, where is that document, does that -- does such a document exist in what you provided the lab?

A All I have is my conversations with the Doctor.

Q Okay. So, it would be accurate to say, that the lab didn't know what your conversations with your Doctor were?

A The Doctor just kept it very simple to

the Lab to know what your private conversations are with your Doctor unless you or your provider share those conversations in some way; correct?

A Correct.

Q Okay. And the documents --

A And I just kept -- we just kept it simple.

Q Okay. But okay. That's fine. But at no point did your Doctor or you submit any documentation from your provider that -- that said he was explicitly telling you that you were precluded from receiving the experimental mRNA vaccines or any other vaccine; correct?

A Correct.

Q Okay. You say in here that your Doctor explicitly told you that you were precluded from taking the experimental mRNA vaccines, the mRNA vaccines were Pfizer and Moderna; correct?

A Correct.

Q Okay. Did your Doctor tell you explicitly, that you were also precluded from taking the Johnson & Johnson vaccine which is not

BY MR. SCHNEIDER:

Q Let's go back to Exhibit Number 2, this page, the provider form. Isn't it true that nowhere on this form that Dr. Schwartz signed that it says you are explicitly precluded from receiving any vaccine?

A It's a medical verification and exemption form for COVID-19 vaccine requirement. So, it is for COVID vaccines.

Q Okay. But all that says here we talked about this before in the check box that says other, all it indicates here is that you have chronic Lyme disease and Lyme-induced immune dysregulation, you see that?

A Correct.

Q Okay. And you wrote that not Dr. Schwartz; correct?

A I took it verbatim. I did not have -- I wouldn't have known to write that, it was directly from his mouth.

Q Okay. But this document is all that Dr. Schwartz -- that you submitted on behalf of Dr.

temporary -- temporary measure so that the lab could process the accommodation requests?

A Yes.

Q Okay. You also understood that if at whatever point your request was denied, you would have one week from that day to either enter proof of the first dose of the vaccination or face termination; correct?

A I see it here.

Q Okay. That is ended up -- ended up what -- what happened to you; correct?

A Yes.

Q Okay. All right. Let's go to the next one. Recognize this e-mail?

(EXHIBIT 7 MARKED)

A I see it to be here.

BY MR. SCHNEIDER:

Q Okay. So this e-mail here at the top is a -- an e-mail that Ms. Bunda-Lee sent to you on October 11th; correct?

A Yes.

Q Okay. And in this e-mail, Ms. Bunda-Lee

asks that you return the request for personal medical records form; correct?

A Yes.

Q And she tells you specifically here that it will enable an APL's medical officer to consult with your medical provider on any necessary follow-up. Do you see that?

A Yes.

MR. SCHNEIDER: Okay. All right. Why don't we take -- we can -- please go off the record.

THE VIDEOGRAPHER: Please standby. We're going off the record. The time on the video --

(Whereupon, a recess was taken.)

THE VIDEOGRAPHER: We are back on the video record. The time on the video monitor is 12:17 p.m.

BY MR. SCHNEIDER:

Q Okay. Let's look at another exhibit.

(EXHIBIT 8 MARKED)

THE VIDEOGRAPHER: Counsel, your

Q How do you know that?
A -- it doesn't change.
Q How do you know that? You don't --
A Because I've -- I asked him, he said, I've already given all the information that they need.
Q And so you went back after the -- after -- when did you go back and talk to him about that issue, about whether or not he would submit something different? When did that conversation happen?
A As soon as I got the requests and he said that I've given the information that they need to make their decision.
Q So when? When was that?
A Whenever afterwards.
Q After --
A I don't remember the --
Q After October 18th?
A When did I get the -- when did I provide the medical journal articles?
Q I mean, do you recall when you did that?

A I don't remember.

Q Okay. Would you say that it was at that same time?

A It was then and then I asked again and he repeated that he already provided the information that was necessary for them to make the call.

Q That he believed.

A Correct.

Q Okay.

A He's my practitioner, he's the one who knows what is good and what's not. He's the only person that I know that -- I credit my life to him.

Q Did Dr. Schwartz ever offer to speak with the lab?

A I don't recall.

Q Okay. Did Dr. Schwartz ever offer to submit any additional information at all other than the journal articles? We'll get to those.

A He said that was sufficient.

Q Okay. Did he -- did Dr. Schwartz ever tell you, give them my phone number and they can

what your understanding is.

A You are trying to -- trying to be God and act like you knew what Lyme disease was. You -- you -- the people that know how to treat Lyme aren't your everyday doctors. They're the ones who -- it's a horrendous experience. I remember the first doctor I went to they didn't believe it was real. You know, you could actually -- it's real. And all of the censorship of whatever -- all the medical studies and whatnot were censored. So I'm not sure what you are asking for.

Q I'm -- I just want to -- I -- I'm not disputing your condition or any of the struggles you've had with it. And really, what I'm trying to understand is, what is your understanding as to why Dr. Schwartz never communicated with the lab directly about why your condition --

A He didn't believe that you would be open to having a real conversation.

Q And so you --

A That you had checked the boxes, and whatever your boxes are, you would have just

fully explains why the vaccination is medically contraindicated for you. Do you see that?

A Yes.

Q And that statement is true; correct?

A According to you.

Q Which part of it is false, according to you?

A Again, I believe my doctor provided all the information that you needed in order to make a decision. I still believe that.

Q Okay. That's your belief?

A That's what I said.

Q Okay.

A It's my belief.

Q But this sentence actually asked for updated medical information, did you not provide -- It is true that you didn't provide any updated medical information from your provider that more fully explains why the vaccination is medically contraindicated for you; correct?

A I provided the blood tests after that.

Q Right, but that blood test, you've

already testified it wouldn't show why you were medically -- why or not you were medically -- you know, medically contradicted.

A And I did. It showed the CD57. It shows that my immune system is at issue where if I add antigens, it would cause havoc in my system.

Q Does it say on the blood work?

A That is what CD57 is.

Q I'm --

A It's an indicator.

Q I'm asking you though, on that blood work --

A It is what it is.

Q Sure. That you would agree that that document speaks for itself; correct?

A Correct.

Q That the information contained therein is exactly what is in there; correct?

A Correct.

Q And nothing more and nothing less?

A Well, I gave the explanation along with it in the e-mail that I sent --

Q Sure.
A -- of my understanding from my doctors of what CD57 means.
Q You're not -- you testified earlier, you're not a medical professional --
A Correct.
Q -- and you're not a doctor
A I'm just saying, this is what I was told by my medical providers.
Q So who said --
A Is all I'm saying.
Q Who told you, did Dr. Sivieri?
A Dr. Sivieri told me that when I first went to him.
Q I mean, let me just -- let me get my question because I think we're talking over each other. Let me just make sure -- at the time you took that updated lab work, did Dr. Sivieri give you an opinion, one way or another about that number?
A I was not able to get my appointment with him. He had explained to me what CD57 was

A That's because --

Q -- documents.

A -- we're hoping that people that read the lab results understand why they had that marker. They assume they don't give you a textbook. They know that once you have that count, and the people that are the Lyme doctors that actually transform people's lives, like me, know that. And that's why I go to that doctor and I don't go to APL's lawyers to save my life. I go to my medical provider.

Q Okay. So, we'll look at that document together in a little bit. And the next sentence here says, additional, you never submitted a signed request for medical records or submitted as signed requests for medical records, it would have allowed APL's medical officer to follow-up directly with your provider to secure further clarification. That's correct? That's a true statement?

A Correct.

Q Okay. On November 22nd, 2021, you met

with an accommodations coordinator who reviewed and verified your request. Do you recall that?

A I -- I do recall that phone call.

Q Okay. Who did you meet with?

A I was in Dan Dockery's admin office on the phone and whoever she was was showing that she did not understand things. And it was not something that was -- it was clear that she would not understand things.

Q Let me ask you this: Is it your position in your -- in this case that the lab should have just accepted your -- well, you know, your -- your own recommendation about what you -- whether or not you were to be accommodated under policy?

A Yes.

Q Okay. And it's your position, in this case, that they should have just -- without speaking with your medical provider or learning any more specific information other than what you provided yourself, that they should have just granted the accommodation to you based on your word?

A People knew me and what I was about was quality -- quality and documentation. Quality with helping people out, and -- yes.

Q Let's go to the next page of this document. First sentence, it says, Therefore, APL's accommodations coordinator has determined that your medical accommodation request is denied at this time. Do you see that?

A Yes.

Q Was it your understanding that you could have submitted additional information even after this which may have changed the result?

A I did submit -- you were in love with blood tests, so I gave the updated CD 57 count. It showed the immune dysregulation an improvement, definitely. Because I've done a lot of hard work to keep my health.

Q Let's go to the next exhibit. Recognize this e-mail?

(EXHIBIT 13 MARKED)

A Yes.

BY MR. SCHNEIDER:

I'm reading. And -- and like you want me to come up with something that I -- I don't understand. I explained to the best of my ability where I was and how I listened to my doctor.

Q Okay. Look -- just looking at the document, you testified earlier that they denied your request because you didn't -- you didn't establish the underlying diagnosis. That's not true. It doesn't say that there; correct?

A I'm seeing one part having to do with submitted a nine-year-old medical documentation. I'm seeing other sentences as well.

Q Okay. Point out to me the sentences that say that your accommodation request was denied because they -- the lab didn't believe that you had Lyme disease? Tell me -- tell me where?

A It said that you are looking for some way to: One, prove that I had Lyme, and two, prove something that is not contraindicated. I don't understand what -- what -- what it is that you're asking me.

Q You're looking at the sentence here that

says, on October 18th -- it says right here, why the COVID vaccine is contraindicated for your diagnosis of Lyme disease. Your diagnosis.

A Right.

Q So --

A And I provided the --

Q But isn't it --

A -- the articles that the doctor provided.

Q But --

A So I -- yeah, I don't understand.

Q Okay. Isn't it true that that sentence indicates that the lab is acknowledging that you've had a diagnosis of Lyme disease? They refer to it as your diagnosis; isn't that true?

A Right.

Q Right.

A But you -- but you're not acknowledging the fact that you still have it. Like, when you have it. You still have it.

Q Okay. But that isn't what -- never mind. Let's move on. I think we've -- we've

say. It's -- it's like how important is the job that I love as opposed to having no health to be able to have the job. I had to make the decision. Am I going to follow the advice of my provider, my -- the doctor, who saved my life? I will go with him every time.

Q Let me ask you this: You sought out Dr. Sivieri and Dr. Schwartz because they're specialists in these areas; correct?

A Correct.

Q And you understand that doctors often consult with one another because they don't -- they might not have specific expertise having to do with any one condition or another; correct?

A Correct.

Q Okay. And tell me what your understanding is about who Hopkins Chief Medical Officer is. Tell me -- tell me, what do you know about his expertise?

A I believe he's in public health.

Q Okay.

A He's just -- his -- his degree and that

he's a senator in charge of vaccine distribution from Maryland.

Q Okay. And do you have any understanding about whether or not he's an autoimmune expert or he's a Lyme's expert, or he's a immuno dysregulation expert?

A No.

Q Okay. Let me follow up question then. You recognize that it's helpful for doctors to consult with one another?

A Yes.

Q And so -- but here you -- you did not sign the form which would have allowed the lab's doctor to speak directly with your doctor; correct?

A Basically, my understanding is from people that know Lyme couldn't believe that someone would -- would try to take their livelihood away that he had the information that he needed. I -- I -- that's -- I put in an ADA accommodation request. I don't understand why you had to be the ones to determine whether my doctor

was a legitimate or whatever. I don't understand.

Q So it's your -- okay. But that doesn't really answer my question. My question was, you didn't sign the form which would have allowed two doctors, two medical professionals, to speak with one another about your specific condition; correct?

A Right, I thought you had the information that you needed.

Q Okay. But the lab was asking for more, and so you knew, at least at that point, that they didn't believe they had enough information; correct?

A There was no information that you can literally provide. Lyme disease isn't something that you, you know -- you show a -- an image or whatever. It -- it -- that -- you cannot provide that. You have to either trust or not.

Q But you don't know whether or what Dr. Schwartz would have said to the labs doctor about your condition. You don't know because it never happened; correct?

Plaintiff's Deposition Exhibit 2

From:	Tarquinio, Sally W.
Sent:	Friday, October 1, 2021 1:55 PM
To:	+Accommodations Coordinator List; Billups, Amy J.
Cc:	Tarquinio, Sally W.
Subject:	Medical Verification for Exemption from Covid-19 Vaccine Rqmt AND Request for Medical Accommodation
Attachments:	S Tarquinio_EmployeeMedicalAccommodation Rqst_211001.pdf; S Tarquinio_MedicalVerificationForm - COVID-19.pdf

Please find attached:

- Medical Verification for Exemption from Covid-19 Vaccination Requirement
- Employee Request for Medical Accommodation

(Note: Raissa Kirk indicated scans of documents was sufficient.)

Let me know if you need anything else.

R/ Sally Tarquinio

EMPLOYEE REQUEST FOR MEDICAL ACCOMMODATION

JHU/APL Sensitive-Restricted

You may request one or more reasonable accommodations to enable you to perform the essential functions of your job by completing the form below and returning it to your group supervisor (or equivalent) and APL's Accommodation Coordinator at Accommodations-Coordinator@jhuapl.edu. In the event that the Laboratory needs to obtain from you and/or your physician(s) information regarding the condition(s) for which you are seeking reasonable accommodation, you will be asked to supply such information and/or asked to provide written authorization for the Laboratory's Medical Office to contact your physician(s).

Employee Name: Sally Tarquinio

Dept/Sect and Group: AMDS1A2B

Work Location: Laurel, MD

Extension: 82683

Group Supervisor: Amy Billups

Group Supervisor's Ext.: 87825

Classification/Job Title: Senior Professional II

Date of Request: 10/11/2021

Please complete each of the questions below.

1. What accommodation(s) are you requesting? Exemption from Covid-19 vaccine requirement and covid-19 testing.

2. Is the accommodation(s) you are requesting temporary or permanent? Permanent

If temporary, state the expected duration the accommodation is needed:

3. How will the accommodation(s) requested enable you to perform your job responsibilities?

To remain healthy & given the medical verification for exemption from covid-19 vaccine requirement & submitted. Immune dysregulation is an excessive immune activation from the years of chronic Lyme Disease, similar to autoimmune disease where I overreact against my body's tissues due to the years trying to kill off the Lyme spirochetes. If I introduce another antigen, my body will go crazy due to immune chaos, and most likely a very bad outcome.

10/11/2021
Date

Sally W. Tarquinio
Employee Signature, if mailing

Provide a completed copy of this form to your group supervisor (or equivalent) and send the original to APL's Accommodations Coordinator at Accommodations-Coordinator@jhuapl.edu.

JOHNS HOPKINS
APPLIED PHYSICS LABORATORY

MEDICAL VERIFICATION FOR EXEMPTION FROM COVID-19 VACCINATION REQUIREMENT

PLEASE PRINT THE FOLLOWING INFORMATION:

Name: SALLY TARQUINIO Date of Birth: Redacted

E-mail: sally.tarquinio@jhuapl.edu Phone Number: Redacted

Department/Sector: AMDS Group: AMDS/AZB

Dear Health Care Provider (MD, NP, DO, PA):

Johns Hopkins University Applied Physics Laboratory (APL) is requiring every staff member to submit proof of at least the first dose of a vaccine by October 15, 2021. All those who begin a two-dose vaccination sequence must also submit verification of the second shot by December 1, 2021. This policy will apply as a condition of employment to all staff, including but not limited to full-time, part-time, temporary-on-call, new hires, interns, and remote workers. The above-named person is requesting a reasonable accommodation from this vaccination requirement. A medical exception from COVID vaccination is allowed for certain recognized contraindications (see U.S. Centers for Disease Control's Interim Clinical Considerations for Use of COVID-19 Vaccines (Currently Authorized in the U.S.).

Please complete the form below and return to: Accommodations-Coordinator@jhuapl.edu. Thank you.

The above-named person should not be immunized for COVID for the following reasons (please check all that apply):

☐ History of previous allergic reaction and documentation to indicate an immediate hypersensitivity reaction to the COVID vaccine or a component of the vaccine. **Please attach supporting DOCUMENTATION or MEDICAL RECORDS.**

☐ Treatment of COVID-19 symptoms with monoclonal antibodies or convalescent plasma within the last 90 days. **Please attach supporting DOCUMENTATION or MEDICAL RECORDS.**

☒ Other – Please provide this information in a separate narrative that describes the reason in detail (these requests will be reviewed on a case-by-case basis). – Chronic Lyme Disease + Lyme induced immune dysregulation

Health Care Provider: [illegible] Health Care Provider Phone No.: 215 891 9206

I certify that Sally Tarquinio has the above contraindication and request their medical exception from COVID vaccination.

Health Care Provider Signature: [signature] Date: 9/29/21
(Note: ink signature required – no digital or stamps)

Health Care Provider Medical License No.: [illegible]

Plaintiff's Deposition Exhibit 3

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Patient First Name	Patient Middle Name	Patient SS#	Patient Phone	Total Volume	Age (Y/M/D)	Date of Birth	Sex	Fasting
TARQUINIO	SALLY			Redacted		52/11/10	Redacted	F	No

Account Address
Mark Sivieri MD
10005 Old Columbia Rd P170
COLUMBIA, MD 21046

Patient Address
Redacted

Additional Information
UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

Tests Ordered
HNK1 (CD57) Panel;Lyme, Western Blot, Serum;Comp. Metabolic Panel (14);Urinalysis, Routine;IgG, Subclasses(1-4);EBV Acute Infection Antibodies;Iron and TIBC;Bartonella Antibody Panel;Lyme, IgM, Early Test/Reflex;Candida Antibodies IgG,IgA,IgM;H pylori, IgM, IgG, IgA Ab;WA1 IgG Antibody, IFA;Thyroxine (T4) Free, Direct, S;DHEA-Sulfate;TSH;Calcitriol(1,25 di-OH Vit D);Histamine Determination, Blood;Vitamin D, 25-Hydroxy;Anti-DNase B Strep Antibodies;HHV 6 IgG Antibodies;Homocyst(e)ine, Plasma;Sedimentation Rate-Westergren;Vitamin B12;Copper, Serum;Zinc, Plasma or Serum;Ferritin, Serum;Antistreptolysin O Ab;C-Reactive Protein, Quant;Triiodothyronine,Free,Serum

General Comments
PID:
A courtesy copy of this report has been sent to the patient.

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
HNK1 (CD57) Panel					
% CD8-/CD57+ Lymphs	2.2		%	2.0-17.0	01
This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. Results of this test are for investigational purposes only. The result should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.					
Abs.CD8-CD57+ Lymphs	**29**	**Low**	/uL	60-360	01
This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. Results of this test are for investigational purposes only. The result should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.					
WBC	4.4		x10E3/uL	4.0-10.5	01
RBC	4.52		x10E6/uL	3.77-5.28	01
Hemoglobin	12.4		g/dL	11.1-15.9	01
Hematocrit	39.4		%	34.0-46.6	01
MCV	87		fL	79-97	01
MCH	27.4		pg	26.6-33.0	01
MCHC	31.5		g/dL	31.5-35.7	01

EXHIBIT 11/16/23 Tarquinio 03
Planet Depos, LLC

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Account Address
TARQUINIO	Mark Sivieri MD 10005 Old Columbia Rd P170 COLUMBIA, MD 21046
Patient First Name: SALLY / Patient Middle Name:	
Patient SS# / Patient Phone: Redacted / Total Volume	
Age (Y/M/D): 52/11/10 / Date of Birth: Redacted / Sex: F / Fasting: No	
Patient Address: Redacted	Additional Information: UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
RDW	15.5	High	%	12.3-15.4	01
Platelets	250		x10E3/uL	140-415	01
Neutrophils	63		%	40-74	01
Lymphs	28		%	14-46	01
Monocytes	6		%	4-13	01
Eos	2		%	0-7	01
Basos	1		%	0-3	01
Neutrophils (Absolute)	2.8		x10E3/uL	1.8-7.8	01
Lymphs (Absolute)	1.3		x10E3/uL	0.7-4.5	01
Monocytes(Absolute)	0.3		x10E3/uL	0.1-1.0	01
Eos (Absolute)	0.1		x10E3/uL	0.0-0.4	01
Baso (Absolute)	0.0		x10E3/uL	0.0-0.2	01
Immature Granulocytes	0		%	0-2	01
Immature Grans (Abs)	0.0		x10E3/uL	0.0-0.1	01
Lyme, Western Blot, Serum					
Lyme Ab IgG by WB:					01
IgG P93 Ab.	Absent				01
IgG P66 Ab.	Absent				01
IgG P58 Ab.	Absent				01
IgG P45 Ab.	Absent				01
IgG P41 Ab.	Absent				01
IgG P39 Ab.	Absent				01
IgG P30 Ab.	Absent				01
IgG P28 Ab.	Absent				01
IgG P23 Ab.	Absent				01
IgG P18 Ab.	**Present**	**Abnormal**			01
Lyme IgG WB Interp.	Negative				01
	Positive: 5 of the following Borrelia-specific bands: 18,23,28,30,39,41,45,58, 66, and 93.				
	Negative: No bands or banding patterns which do not meet positive criteria.				
Lyme Ab IgM by WB:					01

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rec
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Account Address
TARQUINIO	Mark Sivieri MD
Patient First Name: SALLY / Patient Middle Name:	10005 Old Columbia Rd P170
Patient SS# / Patient Phone: Redacted / Total Volume:	COLUMBIA, MD 21046
Age (Y/M/D): 52/11/10 / Date of Birth: Redacted / Sex: F / Fasting: No	
Patient Address: Redacted	Additional Information: UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
IgM P41 Ab.	Absent				01
IgM P39 Ab.	Present	Abnormal			01 ←
IgM P23 Ab.	Absent				01
Lyme IgM WB Interp.	Negative				01

Note: An equivocal or positive EIA result followed by a negative Western Blot result is considered NEGATIVE. An equivocal or positive EIA result followed by a positive Western Blot is considered POSITIVE by the CDC.

Positive: 2 of the following bands: 23,39 or 41
Negative: No bands or banding patterns which do not meet positive criteria.
Criteria for positivity are those recommended by CDC/ASTPHLD.
p23=Osp C, p41=flagellin

Note:
Sera from individuals with the following may cross react in the Lyme Western Blot assays: other spirochetal diseases (periodontal disease, leptospirosis, relapsing fever, yaws, and pinta); connective autoimmune (Rheumatoid Arthritis and Systemic Lupus Erythematosus and also individuals with Antinuclear Antibody); other infections (Rocky Mountain Spotted Fever; Epstein-Barr Virus, and Cytomegalovirus).

Comp. Metabolic Panel (14)

Glucose, Serum	92	mg/dL	65-99	01
BUN	9	mg/dL	6-24	01
Creatinine, Serum	0.70	mg/dL	0.57-1.00	01
eGFR If NonAfricn Am	100	mL/min/1.73	>59	01
eGFR If Africn Am	115	mL/min/1.73	>59	01
BUN/Creatinine Ratio	13		9-23	01
Sodium, Serum	141	mmol/L	134-144	01
Potassium, Serum	3.8	mmol/L	3.5-5.2	01
Chloride, Serum	104	mmol/L	97-108	01
Carbon Dioxide, Total	22	mmol/L	20-32	01
Calcium, Serum	9.1	mg/dL	8.7-10.2	01

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Account Address
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Patient First Name: SALLY / Patient Middle Name	
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Age (Y/M/D): 52/11/10 / Date of Birth: Redacted / Sex: F / Fasting: No	
Patient Address: Redacted	Additional Information: UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
Protein, Total, Serum	7.0		g/dL	6.0-8.5	01
Albumin, Serum	4.5		g/dL	3.5-5.5	01
Globulin, Total	2.5		g/dL	1.5-4.5	01
A/G Ratio	1.8			1.1-2.5	01
Bilirubin, Total	0.5		mg/dL	0.0-1.2	01
Alkaline Phosphatase, S	55		IU/L	25-150	01
AST (SGOT)	19		IU/L	0-40	01
ALT (SGPT)	15		IU/L	0-40	01
Urinalysis, Routine					
Urinalysis Gross Exam					01
Specific Gravity	1.005			1.005-1.030	01
pH	6.5			5.0-7.5	01
Urine-Color	Yellow			Yellow	01
Appearance	Clear			Clear	01
WBC Esterase	Negative			Negative	01
Protein	Negative			Negative/Trace	01
Glucose	Negative			Negative	01
Ketones	Negative			Negative	01
Occult Blood	**3+**	**Abnormal**		Negative	01
Bilirubin	Negative			Negative	01
Urobilinogen,Semi-Qn	0.2		EU/dL	0.0-1.9	01
Nitrite, Urine	Negative			Negative	01
Microscopic Examination	See below:				01
WBC	0-5		/hpf	0 - 5	01
RBC	**11-30**	**Abnormal**	/hpf	0 - 3	01
Epithelial Cells (non renal)	0-10		/hpf	0 - 10	01
Mucus Threads	Present			Not Estab.	01
Bacteria	Few			None seen/Few	01
IgG, Subclasses(1-4)					
Immunoglobulin G, Qn, Serum	804		mg/dL	700-1600	01
IgG, Subclass 1	**361**	**Low**	mg/dL	422-1292	02
IgG, Subclass 2	336		mg/dL	117-747	02
IgG, Subclass 3	**158**	**High**	mg/dL	41-129	02

Results verified by repeat testing

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
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Patient Last Name	Account Address
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Age (Y/M/D): 52/11/10 / Date of Birth: Redacted / Sex: F / Fasting: No	
Patient Address: Redacted	Additional Information: UPIN: 102791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		102791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
IgG, Subclass 4	23		mg/dL	1-291	02
EBV Acute Infection Antibodies					
EBV Ab VCA, IgM	<0.2		AI	0.0-0.8	01
		Negative	<0.9		
		Equivocal	0.9 - 1.0		
		Positive	>1.0		
EBV Early Antigen Ab, IgG	0.3		AI	0.0-0.8	01
		Negative	<0.9		
		Equivocal	0.9 - 1.0		
		Positive	>1.0		
EBV Ab VCA, IgG	5.1	**High**	AI	0.0-0.8	01
		Negative	<0.9		
		Equivocal	0.9 - 1.0		
		Positive	>1.0		
EBV Nuclear Antigen Ab, IgG	>8.0	**High**	AI	0.0-0.8	01
		Negative	<0.9		
		Equivocal	0.9 - 1.0		
		Positive	>1.0		
Interpretation:					01

EBV Interpretation Chart

Interpretation	VCA-IgM	EA-IgG	VCA-IgG	NA-ABS
Susceptible	-	-	-	-
Acute Infection	+	+or-	+or-	-
Convalescent Phase	+or-	+or-	+	+
Chronic or Reactivated	-	+	+	+or-
Old Infection	-	-	+or-	+

\+ Antibody Present - Antibody Absent

Iron and TIBC					
Iron Bind.Cap.(TIBC)	355		ug/dL	250-450	01
UIBC	249		ug/dL	150-375	01
Iron, Serum	106		ug/dL	35-155	01
Iron Saturation	30		%	15-55	01
Bartonella Antibody Panel					
B. henselae IgG	Negative		titer	Neg:<1:320	02

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Account Address
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Age (Y:M:D): 52/11/10 / Date of Birth: Redacted / Sex: F / Fasting: No	
Patient Address: Redacted	Additional Information: UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
B. henselae IgM	Negative		titer	Neg:<1:100	02
B. quintana IgG	Negative		titer	Neg:<1:320	02
B. quintana IgM	Negative		titer	Neg:<1:100	02

Note: Bartonella henselae is now regarded as the etiologic agent of Cat Scratch Disease, bacillary angiomatosis, endocarditis and fever with bacteremia. Bartonella quintana also causes bacillary angiomato-sis particularly among immunocompromised patients, and trench fever.

This test was developed and its performance characteristics determined by LabCorp. It has not been cleared or approved by the Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

Lyme, IgM, Early Test/Reflex

Lyme Disease Ab, Quant, IgM	<0.91		index	0.00-0.90	01
		Negative	<0.91		
		Equivocal	0.91 - 1.09		
		Positive	>1.09		

Note: IgM levels may peak at 3-6 weeks post infection, then gradually decline. FDA currently advises that Western Blot testing be performed following all equivocal or positive EIA results. Final diagnosis should include appropriate clinical findings and a positive EIA which is also positive by Western Blot.

Candida Antibodies IgG,IgA,IgM

Candida Antibodies IgG	<30	U/mL	0-29	02

Results for this test are for research purposes only by the assay's manufacturer. The performance characteristics of this product have not been established. Results should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Account Address
TARQUINIO	Mark Sivieri MD 10005 Old Columbia Rd P170 COLUMBIA, MD 21046

Patient First Name	Patient Middle Name
SALLY	

Patient SS#	Patient Phone	Total Volume
	Redacted	

Age (Y/M/D)	Date of Birth	Sex	Fasting
52/11/10	Redacted	F	No

Patient Address	Additional Information
Redacted	UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
Candida Antibodies IgM	<10		U/mL	0-9	02

Results for this test are for research purposes only by the assay's manufacturer. The performance characteristics of this product have not been established. Results should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.

Candida Antibodies IgA	<10		U/mL	0-9	02

Results for this test are for research purposes only by the assay's manufacturer. The performance characteristics of this product have not been established. Results should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.

H pylori, IgM, IgG, IgA Ab

H. pylori IgG, Abs	<0.9		U/mL	0.0-0.8	01
		Negative	<0.9		
		Indeterminate	0.9 - 1.0		
		Positive	>1.0		
H. pylori, IgA ABS	<0.89		index	0.00-0.88	01
		Negative	<0.89		
		Equivocal	0.89 - 0.99		
		Positive	>0.99		
H.pylori, IgM ABS	<0.80		index	0.00-0.79	01
		Negative	<0.80		
		Equivocal	0.80 - 1.19		
		Positive	>1.19		

Current studies suggest that H. pylori IgM testing should be performed concomitantly with H. pylori IgA and/or IgG tests to support a diagnosis of Helicobacter pylori infection.

For research use only, not for use in clinical diagnostic procedures.

WA1 IgG Antibody, IFA

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Patient First Name	Patient Middle Name
TARQUINIO	SALLY	

Account Address: Mark Sivieri MD
10005 Old Columbia Rd P170
COLUMBIA, MD 21046

Patient SS#	Patient Phone	Total Volume
	Redacted	

Age (Y/M/D)	Date of Birth	Sex	Fasting
52/11/10	Redacted	F	No

Patient Address: Redacted

Additional Information: UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		I02791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
WA1 IgG Antibody, IFA	<1:256				03

REFERENCE RANGE: <1:256

INTERPRETIVE CRITERIA:
<1:256 Antibody not detected
> or = 1:256 Antibody detected

WA1, also known as Babesia duncani, has been associated with symptoms similar to those caused by Babesia microti. Little, if any, crossreactivity occurs between Babesia microti and WA1.

This assay was developed and its performance characteristics have been determined by Focus Diagnostics. Performance characteristics refer to the analytical performance of the test.

Thyroxine (T4) Free, Direct, S					
T4,Free(Direct)	1.47		ng/dL	0.82-1.77	01
DHEA-Sulfate					
DHEA-Sulfate	71.8		ug/dL	35.4-256.0	01
TSH					
TSH	1.090		uIU/mL	0.450-4.500	01
Calcitriol(1,25 di-OH Vit D)					
Calcitriol(1,25 di-OH Vit D)	129.9	High	pg/mL	10.0-75.0	02
Results verified by repeat testing					
Histamine Determination, Blood					
Histamine Determination, Blood	69		ng/mL	12-127	02

Results for this test are for research purposes only by the assay's manufacturer. The performance characteristics of this product have not been established. Results should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.

Vitamin D, 25-Hydroxy

LabCorp
Laboratory Corporation of America

Specimen Number	Patient ID	Control Number	Account Number	Account Phone Number	Rte
182-436-2446-0		10349860468	19516220	410-312-5280	

Patient Last Name	Account Address
TARQUINIO	Mark Sivieri MD
Patient First Name: SALLY / Patient Middle Name	10005 Old Columbia Rd P170
Patient SS# / Patient Phone: Redacted / Total Volume	COLUMBIA, MD 21046
Age (Y/M/D): 52/11/10 / Date of Birth: Redacted / Sex: F / Fasting: No	
Patient Address: Redacted	Additional Information: UPIN: I02791

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
06/30/12 08:41	06/30/12	07/03/12 22:05ET	SIVIERI, M		102791

TESTS	RESULT	FLAG	UNITS	REFERENCE INTERVAL	LAB
Vitamin D, 25-Hydroxy	80.5		ng/mL	30.0-100.0	01

Vitamin D deficiency has been defined by the Institute of Medicine and an Endocrine Society practice guideline as a level of serum 25-OH vitamin D less than 20 ng/mL (1,2). The Endocrine Society went on to further define vitamin D insufficiency as a level between 21 and 29 ng/mL (2).

1. IOM (Institute of Medicine). 2010. Dietary reference intakes for calcium and D. Washington DC: The National Academies Press.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. JCEM. 2011 Jul; 96(7):1911-30.

Anti-DNase B Strep Antibodies

Anti-DNase B Strep Antibodies	<76		U/mL	0-120	02

Results verified by repeat testing
Limit of assay detection is <76

HHV 6 IgG Antibodies

HHV 6 IgG Antibodies	2.05	High	index		02

Negative	<0.76
Equivocal	0.76 - 0.99
Positive	>0.99

Results for this test are for research purposes only by the assay's manufacturer. The performance characteristics of this product have not been established. Results should not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.

Homocyst(e)ine, Plasma

Homocyst(e)ine, Plasma	4.9		umol/L	0.0-15.0	01

Sedimentation Rate-Westergren

Sedimentation Rate-Westergren	2		mm/hr	0-40	01

Vitamin B12

Vitamin B12	977	High	pg/mL	211-946	01

Copper, Serum

Plaintiff's Deposition Exhibit 4

From: Bunda-Lee, Elizabeth A.
Sent: Friday, October 1, 2021 6:03 PM
To: Tarquinio, Sally W.
Cc: +Accommodations Coordinator List; Billups, Amy J.
Subject: Medical Accommodation Request – Tarquinio (Sally)—Acknowledgment
Signed By: Elizabeth.Bunda-Lee@jhuapl.edu

Hello Sally,
This will acknowledge receipt of your accommodation request. We will move forward in working directly with your leadership on occupational development, so we can understand your job requirements. We will get back to you as soon as we have or need additional information. There will be no interim status updates.

To avoid oversight or delay, please continue to direct all correspondence about your request to the Accommodations Coordinator general email address. Because we are processing a large volume of requests, we appreciate your patience with the process.

Very Respectfully,
Liz Bunda-Lee

Liz Bunda-Lee
EEO/ADA Office
office: 443-778-6155
elizabeth.bunda-lee@jhuapl.edu

From: Tarquinio, Sally W. <Sally.Tarquinio@jhuapl.edu>
Sent: Friday, October 1, 2021 1:55 PM
To: Accommodations Coordinator <Accommodations-Coordinator@jhuapl.edu>; Billups, Amy J. <Amy.Billups@jhuapl.edu>
Cc: Tarquinio, Sally W. <Sally.Tarquinio@jhuapl.edu>
Subject: Medical Verification for Exemption from Covid-19 Vaccine Rqmt AND Request for Medical Accommodation

Please find attached:
- Medical Verification for Exemption from Covid-19 Vaccination Requirement
- Employee Request for Medical Accommodation

(Note: Raissa Kirk indicated scans of documents was sufficient.)

Let me know if you need anything else.

R/ Sally Tarquinio

Plaintiff's Deposition Exhibit 6

From: Bunda-Lee, Elizabeth A.
Sent: Monday, October 11, 2021 1:08 PM
To: Tarquinio, Sally W.
Cc: +Accommodations Coordinator List
Subject: Medical Accommodation Request – Tarquinio (Sally)—Adjudication Status/Deadline
Signed By: Elizabeth.Bunda-Lee@jhuapl.edu

Hello Sally,

The Accommodations Team received an overwhelming volume of requests for exemption from the vaccination policy. We understand you may be concerned about the status of your request or what will occur if your accommodation is not approved by October 15th. The Lab's FAQ's address this concern as follows:

> **What happens if I submit my accommodation request on Oct. 1 but my accommodation is not approved by October 15th?**
> Staff members must submit their accommodation request by Oct. 1. Staff members who submitted a request by that date, are cooperating fully and in a timely fashion with the ADA Office, and who have not received a decision on their request by Oct. 15 will be asked to provide a negative COVID-19 test result from the past 72 hours in order to gain access to APL facilities. If the accommodation is denied by the ADA office, the staff member will have one week from the date of the decision to enter proof of their first dose of vaccination into VVS.

It is our understanding that COVID tests must be professionally administered and that home tests will not be acceptable. Local health authorities offer such testing free-of charge, as does APL's health insurance provider, Cigna Allegiance. We will be unable to provide any estimated timelines or interim status updates and appreciate your continued patience with the process.

Very Respectfully,
Liz Bunda-Lee

Liz Bunda-Lee
Deputy Accommodations Coordinator
office: 443-778-6155
elizabeth.bunda-lee@jhuapl.edu

From: Tarquinio, Sally W. <Sally.Tarquinio@jhuapl.edu>
Sent: Monday, October 11, 2021 9:39 AM
To: Accommodations Coordinator <Accommodations-Coordinator@jhuapl.edu>
Cc: Bunda-Lee, Elizabeth A. <Elizabeth.Bunda-Lee@jhuapl.edu>
Subject: RE: Medical Accommodation Request – Tarquinio (Sally)—Acknowledgment

Plaintiff's Deposition Exhibit 7

From: Bunda-Lee, Elizabeth A.
Sent: Monday, October 11, 2021 2:09 PM
To: Tarquinio, Sally W.
Cc: +Accommodations Coordinator List
Subject: Medical Accommodation Request – Tarquinio (Sally)—Medical Development needed - missing a document
Signed By: Elizabeth.Bunda-Lee@jhuapl.edu

Hello Sally,
In reviewing your documents, we are still missing the Request for Personal Medical Records. This form is available here: https://aplweb.jhuapl.edu/insideapl/cll/Pages/ADA.aspx
This will enable APL's Medical Officer can consult with your medical provider on any necessary follow-up.

Very Respectfully,
Liz Bunda-Lee

Liz Bunda-Lee
Deputy Accommodations Coordinator
office: 443-778-6155
elizabeth.bunda-lee@jhuapl.edu

From: Tarquinio, Sally W. <Sally.Tarquinio@jhuapl.edu>
Sent: Monday, October 11, 2021 9:39 AM
To: Accommodations Coordinator <Accommodations-Coordinator@jhuapl.edu>
Cc: Bunda-Lee, Elizabeth A. <Elizabeth.Bunda-Lee@jhuapl.edu>
Subject: RE: Medical Accommodation Request – Tarquinio (Sally)—Acknowledgment

Hi Elizabeth and Accommodations Coordinator,

Please provide the status of my medical accommodation request.

R/ Sally Tarquinio

From: Bunda-Lee, Elizabeth A. <Elizabeth.Bunda-Lee@jhuapl.edu>
Sent: Friday, October 1, 2021 6:03 PM
To: Tarquinio, Sally W. <Sally.Tarquinio@jhuapl.edu>
Cc: Accommodations Coordinator <Accommodations-Coordinator@jhuapl.edu>; Billups, Amy J. <Amy.Billups@jhuapl.edu>
Subject: Medical Accommodation Request – Tarquinio (Sally)—Acknowledgment

Hello Sally,
This will acknowledge receipt of your accommodation request. We will move forward in working directly with your leadership on occupational development, so we can understand

Plaintiff's Deposition Exhibit 8

From: Accommodations Coordinator
Sent: Monday, October 18, 2021 9:26 PM
To: Tarquinio, Sally W.; Accommodations Coordinator
Subject: Medical Accommodations Request Follow up COVID 19 Vaccine

Ms. Tarquino,

The medical documentation you have submitted is over nine years old. Can you please provide current medical documentation from medical provider as to whether the previously stated concerns are still ongoing and why the COVID 19 vaccine is contraindicated with respect to your specific medical condition. Please submit this documentation from your provider no later than November 15, 2021 to the Accommodations Coordinator.

Thank you,

Accommodations Coordinator

Plaintiff's Deposition Exhibit 9

From: Tarquinio, Sally W.
Sent: Thursday, October 14, 2021 4:59 PM
To: Bunda-Lee, Elizabeth A.; McGruder, Shawn S.; Lam, Clarence
Cc: Billups, Amy J.; Uy, Geoffrey S.; Quinn, Stephen J.
Subject: I love my job! - my request for a medical accommodation submitted 1 OCT

After 16 years at JHU/APL, the prospect of not working here is very unsettling. I LOVE MY JOB! I am happy coming to work. I find the complex technical & process challenges fascinating. Identifying gaps, connecting dots, connecting people, helping ensure quality products, helping to create what has never existed before. Uplifting & empowering world-class team members to shine even brighter. The idea of not being connected to APL is horrific to me.

JHU/APL has become a part of my identify. I love my job. It is home. I love the diversity of talent and expertise. The focus on excellence, and ability to delve deep. Yes I've had different positions on different programs, with varying degrees of complexity/innovation. I thrive on programs requiring vision and rigor. I especially love the hypersonics work, joining the team in January 2021. Yes, even with the tight deadlines; the sponsor challenges for quick answers balanced with rigor. I love the Glide Phase Intercept Program team! It has been an honor & joy to support this much needed program, Geoff Uy and his dynamic JHU/APL team, and the larger MDA team. I love my job!

It greatly saddens me that I feel I am being boxed into a corner. To choose my health, or do what I love.

As I indicated in my request for medical accommodation, I have Lyme-induced immune dysregulation. Immune dysregulation is an excessive immune activation from the years of chronic Lyme Disease, where I overreact against my body's tissues due to the years trying to kill off the Lyme spirochetes. If I introduce another antigen, my body will go crazy due to immune chaos, and most likely a bad outcome.

The diagnosis from Dr. Mark Sivieri M.D. (Laurel, MD) that I included with my medical accommodation request was a God-send. Dr. Sivieri spoke of successes he'd witnessed with certain protocols that he was not yet able to offer. Rather than me going to the West Coast for treatment, I started going to the doctor that trains other doctors on these protocols on the East Coast - Dr. Marc Schwartz D.C. (Jennkintown, PA). Dr. Schwartz's patients typically see 27 doctors, before finding him. Dr. Schwartz had over a 10-month waiting list at that time (same with Dr. Sivieri), with an MD on staff that administered some of the protocols. I also saw Dr. Sivieri several times a year, until it became clear that my protocol (a combination from both doctors) works, and is a life-long protocol for me. I continue to see Dr. Schwartz for a regular checkup.

After 16 years, this is what I hope is a solution for all concerned, while we find ourselves in "rough waters".

- A weekly CDC-approved saliva Covid-19 test administered on-site at a quality facility that I pay out of my own pocket.
- Work on-site for 2 to 5 days a week, and at home the other days.

In gratitude for the opportunities that I've had, and going forward at JHU/APL,

R/ Sally Tarquinio

Plaintiff's Deposition Exhibit 10

From: Tarquinio, Sally W.
Sent: Monday, November 15, 2021 4:13 PM
To: +Accommodations Coordinator List
Cc: Billups, Amy J.; Uy, Geoffrey S.
Subject: Re: Medical Accommodations Request Follow-up
Attachments: Evidence of Borrelia autoimmunity.docx; 1-s2.0-S1198743X14628871-main.pdf

Regarding your request for additional medical evidence related to my medical accommodations requests:

As indicated in my 14 October email, I love APL and cannot imagine not working at APL - not helping the government develop a defense against hypersonics. It is my identity. What brings me immense satisfaction & joy. So I felt great relief & happiness when I found out my medical accommodations request was still being reviewed; still a member of the Glide Phase Intercept team. I could breathe. This last week, feeling like I am sleeping on a bed of nails, tremendous stress. Unable to breathe. Hopeful that the highest integrity prevails.

My concerns:

- Dishearted that I may still be at a crossroads between my career and health, given your request for additional medical evidence to support my accommodation requests due to Lyme-induced immune dysregulation.
    1. Lyme Disease (LD) does not go away.
    2. It is "managed" via diet, remedies, nutrition, detoxification, care not to introduce new antigens (for life)... a strict maintenance protocol. Otherwise LD flares back up, and why I am in terror of jeopardizing my hard-won health – and of having to live through another 10-years of horror and significant out-of-pocket expense. This time with the added concern of "immune dysregulation", an excessive immune activation from the years of chronic Lyme Disease, where I overreact against my body's tissues due to the years trying to kill off the Lyme spirochetes. If I introduce another antigen, my body will go crazy due to immune chaos, and most likely a bad outcome.)
        1. Note: I had received a clean bill of health from a much less severe case of LD in 2004, working with a doctor and acupuncturist for over a year. No mention of a maintenance protocol to keep LD in check. Yet a rude awakening from Dr. Sivieri in 2012 that my "new" health issues that I'd been experiencing (more severe than in 2003/04) was due to chronic LD.
    3. It is rare to have "traditional" evidence of Bourellia and associated coinfections that is LD. Lyme and its coinfections have hundreds and thousands of strains, that typical blood tests do not test for. And why Dr. Marc Schwartz's patients typically have seen 27 doctors prior to finding him. Why Dr. Mark Sivieri was able to provide his diagnosis in June 2012 based on my symptoms and what had helped/not helped me. And later noted his surprise that the blood test indicated Lyme.
    4. Spirochetes also have an affinity for hiding/morphing into cyst forms or move into biofilm communities, which makes diagnosis difficult.
    5. I am happy to show you the red dots on my abdomen that is from Babesia/Bartenella coinfections. I've had them for a decade.
        1. Tiny ruptures in small blood vessels under the surface of the skin which lead to the spots.
        2. I went to a LD conference in 2018 with doctors from across the country, and specifically asked if these dots will ever go away. Doctor's answer: yes, once the infection is gone.

6. Dr. Schwartz provided two journal articles on Lyme-induced immune dysregulation in support my request. (.pdfs attached):
    1. https://pubmed.ncbi.nlm.nih.gov/15695691/
    2. https://pubmed.ncbi.nlm.nih.gov/15214872/

- This medical accommodations request has nothing to do with politics. It is not an irrational fear on my part.
- Lyme Disease took over 10 years out of my life. It was horrific. A life I wouldn't wish on my "worst enemy". Here is some of my journey:
    1. I started having issues with my usually abundant energy and ability to multiplex.
    2. Searching for a diagnosis as numerous doctors, while expensive, did nothing to improve my health.
    3. It was especially hard for me as I live alone, and did not have help from friends or family. Including significant monetary cost and time needed for appointments and treatment.
    4. I found a doctor who initially helped with nutritional deficiencies (infection loves what I thought was my nutrition). I had lost a lot of weight, and started wearing size 0 clothes (from size 10). It hurt to sit on hard surfaces because I had little "padding". The doctor had me taking a lot of remedies, but eventually I started back-sliding.
    5. I found another doctor, who initially focused on weekly physical detoxing and I thankfully felt relief. But eventually that no longer helped. (This was one of the symptoms that later led Dr. Sivieri to a LD diagnosis, as I later learned that detoxification on its own was not sufficient.)
    6. I had been used to doing the work of two to three people, and found I eventually needed to conserve whatever energy I had to perform my duties at work and retain my job.
        1. It had gotten such that I didn't have energy to empty the dishwasher. Let alone my active life of Viennese waltzing, Argentine Tango & Zydeco.
    7. Until one day, I got a mosquito bite that quickly turned into a crazy design. When I did a web-search it, I found a similar image with an urgent note to see a doctor asap. That's when I contacted Dr. Sivieri. However he was booked 10 months out at that time. I was persistent, calling every other day, and three weeks later, the office manager took pity on me and I saw Dr. Sivieri in June 2012.
    8. He was confident based on my symptoms and what had helped & not helped me that I had LD, including other aspects typical to LD. His diagnosis prior to any blood tests was a methylation defect (inability to detoxify toxins), heavy metals/chemical toxicity, mold, EMR sensitivity (which later turned into hypersensitivity due to high levels of heavy metals).
    9. I followed all of Dr. Sivieri's recommendations, and felt only minor improvement.
    10. Dr. Sivieri indicated in confidence that he himself had had success with a particular doctor's protocol, but found his clients were not willing to be dedicated to following the extensive protocols, so he'd started offering more simplistic protocols that likely would not address my specific needs.
    11. So I scheduled an appointment with the doctor he spoke of, even though he is on the West Coast. But quickly realized how costly this would be, and instead sought an appointment with the doctor who taught this protocol on the East Coast (Dr. Marc Schwartz). Dr. Schwartz had well over a 10-month wait list.
    12. So I worked with other respected Lyme doctors in the meantime. Several willing to treat me only if I turned my circuit breakers off at night, so my body could detox.
    13. Eventually my persistence paid off, and I was able to see Dr. Schwartz in 2013.

14. Besides work, my doctor taught me how to made my own liposomal cocktails and tea each day. I was asked to take over 60 remedies, mostly 3x/day. Definitely a full time job. On-site IVs, injections, laser treatments, ... in-home ionic foot baths, saunas, coffee enemas... Slowly but surely I made progress.
15. Focused on being product at work, and letting go of everything not absolutely essential at home so that I could focus on my health during the weekends.
16. I learned I had hypercoagulation when IVs were not feasible without me taking certain remedies and PEMF sessions.
17. At one point, I told Dr. Sivieri that I did not think I could continue working. That it was all too much. But with his & Dr. Schwartz encouragement, I hung in & periodically traveled to Philadelphia for extra treatment if it ever got too bad. And then was good to go until I needed another treatment. Thank God for Dr. Schwartz!
18. I kept Dr. Sivieri up to date until it became clear that my protocol (a combination from both doctors) works, and is a life-long protocol for me.
19. I continue to see Dr. Schwartz for checkups.

- Blessed that I have my "health" back. I've learned what my body needs, and am diligent about giving it to my body. Adhering to my maintenance LD protocol. Keeping LD in the background (symptoms at bay).
    1. I have my abundant energy back. And can physically dance again! (Though not currently on my plate since Covid-19.)
    2. I can easily multiplex again.
    3. Able to work fulltime again, doing a job that I love doing.

- Terrified to introduce the vaccine (and it's antigens) into my body. Terrified that I will end up back where I was ten years ago; facing another decade of extensive treatment (vs simply my maintenance protocol). Or this time, symptoms will be worse; and not respond to treatment. Sad that I may still be boxed into a corner if my medical accommodations request is not granted. To have to choose my health, or do what I love. Baffled that things may come to this.
    1. My doctor is strongly advising me not to take a vaccine, or there will likely be a bad outcome.
    2. Knowing that I may need to sell my home if I choose my health. Lose my career.
    3. Knowing what I know about Guillian-Barre (a known covid-19 vaccine risk).
        1. I joined a group of people who had benefited from a remedy that also greatly helped me. Sharing what worked/not worked. Offering encouragement. Most people had LD; others had other health concerns. At one point, the group had an influx of people with Guillain-Barre, as one started sharing with others with GB how they had been helped by taking this remedy. So I became aware of the horrors of that diagnosis.
    4. Aware of other patients who had Lyme encephalitis and lyme myocarditis.... also known covid-19 vaccine risks.
    5. Having had hypercoagulation (no longer an issue for me), terrified of having to address this known covid-19 vaccine risk again, with the added concern that this known adverse reaction to the vaccine can come on quickly.

6. Also understanding that the FDA-approved vaccine, Comirnaty, is not legally the same as Emergency Use Authorization (EUA) BioNTech, as noted in the FDA's approval letter's footnote 8. There is also no Comirnaty inventory available in the US. Testing is EUA, as are the masks.

Hopeful this helps you better understand what I've gone through with respect to LD, my terror of introducing foreign antigens into my body due to Lyme-induced immune dysregulation, and my request for medical accommodations. I cannot imagine me taking the risk of blowing on embers that are still not put out after over 10 years.

And I cannot imagine the prospect of not working at APL. I LOVE MY JOB! I am happy coming to work. I love the complex technical & process challenges. I love the diversity of talent & expertise, the focus on excellence, and ability to delve deep. I especially love the hypersonics work. I love the Glide Phase Intercept Program team! It's an honor & joy to support this much needed program, Geoff Uy and his dynamic team, and the larger MDA team. I love APL and it has become a part of my identify.

After 16 years at APL, this is what I hope is a solution for all concerned, while we find ourselves in "rough waters".

- Medical exemption from the Covid vaccine.
- Weekly FDA-approved saliva Covid-19 test administered at a quality facility, that enables a full week's entrance into APL facilities as done at many federal & state agencies. (5 days a week working on-site at APL).
- Or work on-site 2 days a week, and at home the other days.

In gratitude for the opportunities that I've had, and going forward at JHU/APL,

R/ Sally Tarquinio

Plaintiff's Deposition Exhibit 11

Evidence of Borrelia autoimmunity-induced component of Lyme carditis and arthritis

Elizabeth S Raveche[1], Steven E Schutzer, Helen Fernandes, Helen Bateman, Brian A McCarthy, Steven P Nickell, Madeleine W Cunningham

Affiliations: Departments of Pathology and Medicine, New Jersey Medical School, University of Medicine and Density of New Jersey; Department of Molecular Genetics and Microbiology, University of New Mexico, University of Oklahoma Health Sciences Center

Evidence of Borrelia autoimmunity-induced component of Lyme carditis and arthritis

- PMID: 15695691
- PMCID: PMC548028
- DOI: 10.1128/JCM.43.2.850-856.2005

Abstract

We investigated the possibility that manifestations of Lyme disease in certain hosts, such as arthritis and carditis, may be autoimmunity mediated due to molecular mimicry between the bacterium Borrelia burgdorferi and self-components. We first compared amino acid sequences of Streptococcus pyogenes M protein, a known inducer of antibodies that are cross-reactive with myosin, and B. burgdorferi and found significant homologies with OspA protein. We found that S. pyogenes M5-specific antibodies and sera from B. burgdorferi-infected mice reacted with both myosin and B. burgdorferi proteins by Western blots and enzyme-linked immunosorbent assay. To investigate the relationship between self-reactivity and the response to B. burgdorferi, NZB mice, models of autoimmunity, were infected. NZB mice infected with B. burgdorferi developed higher degrees of joint swelling and higher anti-B. burgdorferi immunoglobulin M cross-reactive responses than other strains with identical major histocompatibility complex (DBA/2 and BALB/c). These studies reveal immunological cross-reactivity and suggest that B. burgdorferi may share common epitopes which mimic self-proteins. These implications could be important for certain autoimmunity-susceptible individuals or animals who become infected with B. *burgdorferi*.

Lyme disease is caused by the spirochete *Borrelia burgdorferi* (4, 40). One isolate, B31, has been completely sequenced (15). The bacterium is complex, containing more plasmids than other bacteria, and over 90% of these plasmid genes have no similarity to genes outside *Borrelia* spp. (6, 15). Some of its plasmids are frequently lost during cultivation. Loss of some genes may block experimental in vivo infectivity from one host to another yet may permit pathogen survival even after antibiotic therapy (5). This may allow an inflammatory host response with certain

clinical features that resemble autoimmune manifestations (41). Thus, it is plausible that individual clinical manifestations from an infection may vary depending on the combination of host response and pathogen properties. These factors prompted us to consider experiments to support or counter the possibility of an infection-triggered autoimmunity-like phenomenon in certain cases of Lyme disease, especially when the organism or its antigenic material persists beyond an acute period. We decided to pursue experimentation after our database search revealed common motifs between *B. burgdorferi* and human alpha myosin heavy chain.

There is precedent for infection-triggered autoimmunity or inflammation and a genetic predisposition. Molecular mimicry is one mechanism of autoimmunity following infection (16, 45) and may have relevance to the arthritis and carditis of Lyme disease. Arthritis and autoimmune carditis are common sequelae following infection with *Streptococcus pyogenes* (16, 45, 46). Autoimmune damage and arthritis can also result secondary to infection with *Shigella*, *Salmonella*, and *Yersinia* (24). Arthritis and arthralgia are common manifestations of Lyme disease (38) and are seen in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. It has been postulated that infectious agents may trigger and sustain chronic inflammatory arthritis due to the persistent release or presentation of immunogenic material in affected individuals (2, 3, 20, 32). Alternatively, arthritis may be due to the triggering of anti-self-reactivity from cross-reactive antibodies (Abs) which recognize both the infectious agent and self-components. Both may occur.

In this study, we used the murine NZB model of autoimmune disease for experimental *B. burgdorferi* infection to determine if it provokes demonstrable features such as joint swelling and anti-*B. burgdorferi* antibodies which bind to self-components. We also investigated whether antistreptococcal monoclonal Abs (MAbs) that react with streptococcal M proteins and self-components also react with *B. burgdorferi*-specific proteins.

Figures

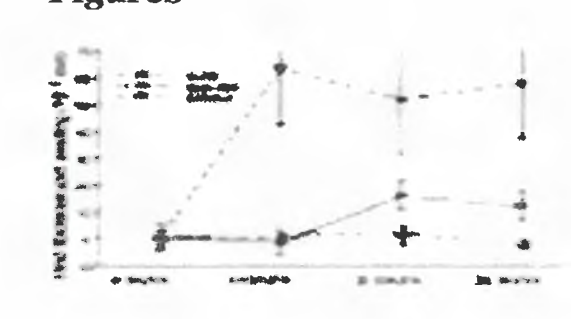

FIG. 1.

Joint swelling in mice at...

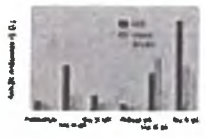

FIG. 2.

Levels of anti- *B. burgdorferi*...

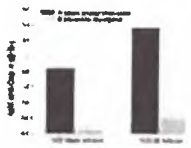

FIG. 3.

Levels of IgM anti-OspA antibodies...

FIG. 4.

The mean ELISA OD (O.D.)...

FIG. 5.

Levels of IgM anti- *S*....

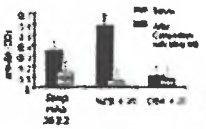

FIG. 6.

Competition of anti- *B. burgdorferi*...

All figures (7)

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ABSTRACT

We investigated the possibility that manifestations of Lyme disease in certain hosts, such as arthritis and carditis, may be autoimmunity mediated due to molecular mimicry between the bacterium *Borrelia burgdorferi* and self-components. We first compared amino acid sequences of *Streptococcus pyogenes* M protein, a known inducer of antibodies that are cross-reactive with myosin, and *B. burgdorferi* and found significant homologies with OspA protein. We found that *S. pyogenes* M5-specific antibodies and sera from *B. burgdorferi*-infected mice reacted with both myosin and *B. burgdorferi* proteins by Western blots and enzyme-linked immunosorbent assay. To investigate the relationship between self-reactivity and the response to *B. burgdorferi*, NZB mice, models of autoimmunity, were infected. NZB mice infected with *B. burgdorferi* developed higher degrees of joint swelling and higher anti-*B. burgdorferi* immunoglobulin M cross-reactive responses than other strains with identical major histocompatibility complex (DBA/2 and BALB/c). These studies reveal immunological cross-reactivity and suggest that *B. burgdorferi* may share common epitopes which mimic self-proteins. These implications could be important for certain autoimmunity-susceptible individuals or animals who become infected with *B. burgdorferi*.

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In this study, we used the murine NZB model of autoimmune disease for experimental *B. burgdorferi* infection to determine if it provokes demonstrable features such as joint swelling and anti-*B. burgdorferi* antibodies which bind to self-components. We also investigated whether antistreptococcal monoclonal Abs (MAbs) that react with streptococcal M proteins and self-components also react with *B. burgdorferi*-specific proteins.

MATERIALS AND METHODS

Mice.

All mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and housed in the animal facilities at the University of Medicine and Dentistry of New Jersey. DBA/2, BALB/c, and NZB mice were employed in this study. Mice (five mice in a group) were infected with *B. burgdorferi*, and sera were obtained at various time points following infection. Joint swelling of tibiotarsal joints was assessed weekly. At the time of death, sera were obtained by retro-orbital sinus puncture, and tissues were saved for histopathological examination.

Sequence analysis of protein similarities between OspA of *B. burgdorferi* and M proteins of *S. pyogenes*.

GenInfo, a multiple-database information retrieval and analysis system at the National Center for Biotechnology Information, was used to reexamine previously noted sequence homologies between *B. burgdorferi* lipoproteins and *S. pyogenes* M proteins (30). Amino acid sequence comparisons between OspA (GenPept accession number AAC6620) and *S. pyogenes* M protein sequences in the Swiss-Prot, GenPept, and PIR databases were performed by using FASTA software homology comparisons (31). LFASTA was used to determine multiple similarities between *B. burgdorferi* OspA and *S. pyogenes* M5 sequences which had known cross-reactivity with self-proteins (8). Inclusion of conservative amino acid changes (amino acid substitutions that share the same properties) increased the overall similarities between these sequences. Peptides derived from the primary structure of the M5 protein were chemically synthesized for use as experimental controls with a DuPont RAMPS manual peptide synthesizer as previously described (19), and the high-performance liquid chromatography-purified synthetic peptide was confirmed by amino acid analysis. The peptide sequences were NT2 (KEALDKYELENHDLKTKN), NT3 (LKTKNEGLKTENEGLKTE), and NT4 (GLKTENEGLKTENEGLKTE).

Borrelia strain and infections.

The low-passage *B. burgdorferi* isolate 910255 used in these experiments has previously been described (23). Spirochetes were grown in vitro in Barbour-Stoenner-Kelly medium (1), harvested, and counted. Mice were injected subcutaneously with 5×10^5 *B. burgdorferi* spirochetes in a total volume of 0.3 ml.

Joint lesion assessment.

Arthritic joint lesion development in *B. burgdorferi*-infected mice was quantitated by measuring changes in the anterior-posterior tibiotarsal joint diameter over time. Before experimental *B. burgdorferi* infection, baseline joint diameters for all mice were measured and recorded. Measurement of joint swelling in *B. burgdorferi*-infected mice was performed at weekly intervals by using a spring-loaded microcaliper (Federal, Providence, R.I.). Data are reported as the difference in diameter from the uninfected time zero with each animal serving as its own control.

ELISA.

Enzyme-linked immunosorbent assays (ELISAs) were performed by using Immulon plates according to established protocol with minor modifications. Briefly, plates were coated with antigens at 10 μg/ml. Antigens employed were *S. pyogenes* M5 protein (obtained as described previously [12]), bovine myosin (Sigma-Aldrich, St. Louis, Mo.), and sonicated *B. burgdorferi* protein (strain 910255) and OspA (obtained from J. Dunn). Plates were coated with the various antigens for 2 h at room temperature, and 2% bovine serum albumin was used to block the plates. Antistreptococcal MAbs were hybridoma culture supernatants obtained from fusions of BALB/c spleens from mice immunized with purified M type 5 streptococcal membranes as described previously (12, 36). Sera (from mice immunized with *B. burgdorferi*) or MAbs were added to the blocked and washed triplicate wells and incubated for 1 h at 37°C. Sera from *B. burgdorferi*-infected animals were used at a 1:100 dilution. Antistreptococcal MAbs were tested as undiluted culture supernatants. Wells were then washed and incubated for 1 h at 37°C with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) (γ chain specific; Sigma-Aldrich) or conjugated goat anti-mouse IgM (μ chain specific; Sigma-Aldrich). After washing, the plates were developed with ABTS [2,2′azinobis(3-ethylbenzthiazolinesulfonic acid] or TMB peroxidase substrate (Kirkegaard & Perry, Gaithersburg, Md.). Specific Ab concentration was determined by incubation of sera on plates coated with either anti-IgM or anti-IgG, and optical density (OD) readings were compared to standards of known IgM or IgG concentrations. Competitive ELISAs to determine polyspecificity (9) were performed by preincubation of sera or monoclonal antibodies with specific antigens (10 μg/ml) at both 1 h at room temperature and overnight at 37°C prior to addition to the ELISA plates. Nonspecific competition was performed with mouse IgM. Percent inhibition was calculated by comparing the OD readings with values obtained with no preincubation as previously described (9).

Western blot analysis.

B. burgdorferi (B31) sonicates (kindly provided by Marc Golingtly, State University of New York, Stony Brook, N.Y.) or recombinant full-length OspA (expressed in *Escherichia coli* and purified by Triton X-114 and ion exchange chromatography as described previously [11]) was added to Laemmli gel sample reducing buffer consisting of 2% sodium dodecyl sulfate, 60 mM Tris-HCl (pH 6.8), 0.1 M dithiothreitol, 0.005% bromophenol blue, and 10% glycerol and boiled to load each well of the preparative gel with

either 16 μg of whole *B. burgdorferi* sonicates or 1.6 μg of recombinant OspA protein. Molecular weight standards were run in tandem. The Western immunoblot was run and the blot was developed as previously described (35). Mouse sera were reacted with nitrocellulose strips at a 1:100 dilution. Antistreptococcal MAbs were concentrated 10-fold using a SpeedVac. Secondary Abs consisted of goat biotinylated anti-mouse IgM (μ chain specific) or biotinylated anti-mouse IgG (γ chain specific).

Microscopic examination and image acquisition.

High-quality computerized images were obtained from the hematoxylin- and eosin-stained sections by using a DM-RB compound microscope (Leica Microskopie und System GmbH) and the Image Pro Plus version 4.0 image processing and analysis system for Windows (Media Cybernetics, Silver Spring, Md.). A 10× PL Fluotar lens was used for this application. The images were acquired through a DEI-750 CE color camera (Optronics, Goleta, Calif.), and the digitized images were processed using Microsoft Windows NT Workstation, version 4.0, with a Trinitron Pentium III computer. Images were assessed for the number of nuclei present in a fixed area. The total area of the image was obtained as well, allowing for determination of the amount of nuclear area per total image area. While this method was unable to distinguish between cardiac cells and inflammatory cells, the actual nuclear area of cardiac cells for a given area should be roughly the same in both treated and untreated samples. Once the nuclear area and total area values were obtained, they were exported into Microsoft Excel for statistical analysis.

PCR amplification of *B. burgdorferi* DNA.

PCR amplification and detection of *B. burgdorferi* DNA followed previously reported procedures that have been shown to have high reliability in detection of *B. burgdorferi* DNA in mouse tissues (27). The OspA and OspB primers employed have been shown to have 92 to 96% reliability in detecting *B. burgdorferi* DNA in mouse tissues (27). Primers were as follows: primer set 15/272 (detects a 280-bp portion [positions 15 to 294] of the OspA gene), consisting of OspA6 (ATT GGG AAT AGG TCT AAT ATT AGC CT) and OspA7 (TGT GGT TTG ACC TAG ATC GTC AG); and primer set 1110/1428 (detects a 328-bp portion [positions 1110 to 1438] of the OspB gene), consisting of OspB-1110 (AAA CGC TAA ACA AGA CCT TCC TG) and OspB-1411 (AGC TTT GAG AGT TTC CTC TGT TAT TGA).

RESULTS

Overall, our results show that an autoimmunity-prone NZB mouse can develop a greater degree of clinicopathologic features than a non-autoimmunity-prone mouse. Furthermore, there is a greater antibody response to the inciting infectious pathogen, *B. burgdorferi*, which is cross-reactive with host tissue.

Joint swelling in mice infected with *B. burgdorferi*.

Autoimmune NZB and nonautoimmune mice were infected with *B. burgdorferi*, and tibiotarsal joint swelling was measured (Fig. 1). Joint swelling was moderate in the NZB mice and maximal at 2 weeks postinfection, while nonautoimmune *H-2*-matched control strains showed little joint inflammation. This was significant by the Student's *t* test (<0.05). NZB mice experienced increased joint swelling over the duration of the experiment.

Antibody response in mice infected with *B. burgdorferi*: anti-*B. burgdorferi*, anti-OspA, and anti-*S. pyogenes* M5.

Analysis of the immune reactivity of the sera from all mice infected with *B. burgdorferi* indicated that the IgM anti-*B. burgdorferi* response was maximal on day 20 and that autoimmune NZB mice had the highest levels (Fig. 2). The IgM anti-*B. burgdorferi* response was temporally related to the peak joint swelling observed in these mice. The nonautoimmune strains of mice showed reduced IgM anti-*B. burgdorferi* responses and reduced joint swelling relative to that observed in the *H-2*-identical autoimmune NZB mice (Fig. 2). There was little difference between the NZB and the nonautoimmune mice in terms of IgG response at day 20, when joint swelling was observed in NZB mice, but at day 35, a significant increase in the IgG response in NZB mice was seen.

To further characterize the antibody response to *B. burgdorferi*, the response to a *B. burgdorferi*-specific protein, OspA, was studied. There was a significant increase in anti-OspA reactivity in NZB mice following infection. (Fig. 3). Restated, it appears that there is an inducible IgM-specific component of anti-OspA antibody when the animal is infected. Since only the NZB strain demonstrated the induction of anti-myosin cross-reactivity following infection with *B. burgdorferi*, competitive analysis was performed with this strain. No reactive changes to cardiolipin were observed.

Anti-self-reactivity induced in *B. burgdorferi*-infected mice.

In Fig. 4, the induction of anti-myosin reactivity in mice infected with *B. burgdorferi* is shown. The NZB mice had more anti-myosin reactivity following *B. burgdorferi* infection than did the two nonautoimmune strains tested. In general, NZB strains are the most responsive strains (Fig. 5). Approximately 50% of the reactivity of *S. pyogenes* M MAb (MAb 36.2.2), which was cross-reactive to *B. burgdorferi*, was reduced (Fig. 6). The IgM level of NZB mice at day 20 was competitively reduced by approximately 90% (Fig. 6). The non-autoimmunity-prone strain, DBA, which had only a limited IgM response to *B. burgdorferi* after infection at day 20, had an insignificant reduction of that reactivity. Since anti-myosin reactivity is often generated following *S. pyogenes* infection, we looked for and found a sequence similarity in silico. This similarity was found between *B. burgdorferi* OspA and *S. pyogenes* M5 peptide already associated with cross-reactivity (the region of similarity between OspA and *S. pyogenes* M5 was in the previously identified NT2 region [amino acids 159 to 168]). The induction of anti-*S. pyogenes* M5 IgM antibodies following infection with *B. burgdorferi* was significant only in the autoimmunity-prone NZB mice and not with DBA or BALB/c mice on day 20 and day 35 postinfection (Fig. 5). As noted, in the competitive ELISA, only the NZB anti-*B. burgdorferi* antibodies were significantly decreased (90% reduction) by preincubation with *S. pyogenes* M5, and for comparison, an antistreptococcal MAb, 36.2.2, with known cross-reactivity with myosin is presented (Fig. 6). We found amino acid sequence similarity between *B. burgdorferi* OspA and streptococcal M protein, which suggested that *B. burgdorferi* and *S. pyogenes* share epitopes which cross-react with self-components. This reactivity was investigated further by Western analysis. Infected NZB and DBA/2 mice both demonstrated anti-*B. burgdorferi* IgM reactivity with a band at 31 kDa (marked by recombinant *B. burgdorferi* OspA) (Fig. 7). In addition, the antistreptococcal MAbs recognized this same 31-kDa OspA protein. We used NT3 and NT4 as negative controls. ELISA and Western blot results show that two anti-*S. pyogenes* M5 MAbs, 36.2.2 and 54.2.8, reacted with full *S. pyogenes* M5 but not NT3 or NT4 synthetic subpeptides (Fig. 7).

Carditis following *B. burgdorferi* infection in NZB mice.

Heart tissue was studied for the presence of *B. burgdorferi* spirochetes and inflammation. Infected NZB mice remained positive for the presence of *B. burgdorferi* DNA throughout the duration of the experiment (Table 1). Controls were negative for the presence of spirochetes in the heart as well as inflammation. Microscopic examination of heart tissue showed that inflammation in the heart was readily observed in NZB mice following *B. burgdorferi* infection. This was quantified by enumerating cellular infiltrates (nuclear ratios). A statistically significant increase in cellular infiltration was observed in the *B. burgdorferi*-infected hearts. The computer analysis showed that there were 663 ± 36.9 cells in the control mice compared to 996.8 ± 72.4 cells in the infected mice.

DISCUSSION

The present study investigated the possibility of epitope similarities between the infectious agent (*B. burgdorferi*) responsible for Lyme disease and host proteins. Our experiments took advantage of two well-characterized systems to study self-reactivity. The first system involved the relationship between the M5 protein of *S. pyogenes* and self-components. This relationship was analyzed further since we noted a sequence similarity between the *B. burgdorferi* protein OspA and the *S. pyogenes* M5 protein. The second system used the NZB strain of mice, which develops autoimmune disease characterized by IgM hypergammaglobulinemia due to a subset of B cells, B-1 cells, noted for the polyreactive nature of the IgM they produce. NZB mice were infected with *B. burgdorferi* to determine if cross-reactive antibodies were produced. The anti-*B. burgdorferi* response in NZB mice was compared to that of two nonautoimmune strains of mice, DBA/2 and BALB/c, as well as uninfected NZB mice. All three strains are myosin heavy chain identical, *H-2*^{d}, to control for the fact that the development of Lyme arthritis in humans may be linked to the myosin heavy chain loci (39). We hypothesized that in autoimmunity-prone animals, much of the anti-*B. burgdorferi* response might also recognize the *S. pyogenes* M5 protein as well as myosin due to molecular mimicry. We found that the infected NZB strain developed higher levels of IgM anti-*B. burgdorferi*, which was temporally related to the higher levels of joint swelling observed in NZB mice compared to those of control strains. The IgM anti-*B. burgdorferi* response in NZB also cross-reacted with *S. pyogenes* M and myosin, both of which share sequence homology with *B. burgdorferi* OspA, suggesting a role for molecular mimicry in the generation of these Ab reactivities. These findings are consistent with prior evidence that immune responses to infectious agents may lead to deleterious autoimmune phenomena due to the immunological cross-reactivity of bacterial epitopes and self-components (8, 16).

In this report, sequence similarity between the NT2 peptide of the *S. pyogenes* M5 protein and *B. burgdorferi* OspA was noted. Based on the sequence similarity and secondary structure similarities between OspA, *S. pyogenes* M5 (NT2 peptide), and myosin, the immune response to *B. burgdorferi* which results in an anti-OspA response may have both beneficial and potentially harmful effects. OspA is a major outer surface protein of *B. burgdorferi* and is a basic lipoprotein with an approximate size of 31 kDa and demonstrates antigenic variability (29, 44). In the majority, but not all, of spirochetes involved at the onset of infection, OspA is downregulated, whereas OspC is upregulated (10). However, sufficient exposure occurs, since several studies have detected T- and B-cell responses to OspA in the acute phase (21, 25, 35). Antibodies specific for OspA occur in Lyme disease, and when anti-OspA antibodies are passively transferred, they protect (14, 37). Although antibodies play a role in

resistance to *B. burgdorferi* (22), T cells and their associated cytokines are also involved in the type of immune response invoked during *B. burgdorferi* infections (22, 26, 28). OspA has been shown to cause polyclonal activation of B cells (43), which may result in increased production of polyreactive IgM antibodies which cross-react with self-components in *B. burgdorferi*-infected NZB mice. However, other mechanisms may be involved.

It is possible that cross-reactive antibodies initially triggered by *B. burgdorferi* lipoproteins such as OspA have initiated an autoimmune reaction. The IgM response to *B. burgdorferi* and the ability of *S. pyogenes* M5 to compete with this reactivity further suggest that cross-reactive antibodies induced by *B. burgdorferi* may be involved in mimicry to self-components and could play a role in certain cases of Lyme arthritis. Anti-*B. burgdorferi* antibodies, in particular anti-OspA antibodies, may be protective in Lyme disease; however, even healthy patients vaccinated with an OspA-containing formulation developed transient arthralgia (34). However, a reactive arthritis was not a universal finding in the OspA vaccine recipients (42). In some cases, the anti-*B. burgdorferi* response may have undesirable effects which may be more severe in individuals with a predisposition towards autoimmunity. A component of the anti-*B. burgdorferi* response is T-cell independent (13), and a subpopulation of T-cell-independent *B. burgdorferi*-responsive B cells may produce polyreactive antibodies which cross-react with self-components. In the present study, the fact that the anti-*S. pyogenes* M monoclonal antibodies (which recognized the NT2 peptide) also reacted with *B. burgdorferi* proteins suggests that the polyreactive nature of IgM antibodies elicited in response to both *S. pyogenes* and *B. burgdorferi* may play a role in autoimmunity and the development of arthritis. In addition to sequence similarity between *B. burgdorferi* proteins and *S. pyogenes* M proteins, other infectious agents such as coxsackie B3 virus, known to induce autoimmune damage, also have sequence similarity to *S. pyogenes* M5 (17, 19). These studies indicate that pathogenic immune response to cross-reactive self-epitopes may be responsible for cardiac injury following infection with a number of agents. In this report, we provide evidence that similar to the anti-*S. pyogenes* response, anti-*B. burgdorferi* antibodies may have the ability to cross-react with self-components and lead to autoimmune tissue reactivity or impairment of function.

Studies with non-autoimmunity-prone mice have shown that in the course of an immune response to foreign antigen, autoreactivity arises normally and is eliminated by apoptosis (33). Autoimmunity-prone individuals may have an increase in the production of polyspecific IgM antibodies which cross-react with self-components as well as defective apoptosis induction which would otherwise eliminate self-reactive B cells, including those which have acquired self-reactivity as a result of somatic mutation. This suggests that the basic mechanism leading toward the production of autoantibodies may lead to the induction of cross-reactive antibodies following some infections, as was observed in the present study. It is conceivable that cross-reactive Abs may remain at low circulating levels but may still be tissue bound.

Immunological response to *B. burgdorferi* may be responsible for many of the symptoms associated with Lyme disease, and Ab production may be crucial to clearance of this spirochete. It is unclear which isotype may be more effective in clearance, but Th1 or Th2 subsets determine the predominant immunoglobulin class involved. In patients who have treatment-resistant chronic Lyme disease, autoimmune mechanisms may play a role in persistent disease (18, 41).

We found that the majority of our infected animals were PCR positive for *B. burgdorferi* DNA. Thus, *B. burgdorferi* may be an active agent in causing cardiac damage or dysfunction (7), but the mechanism of the usual human transient conduction defections remains unexplained.

in summary, our data, albeit preliminary, suggest that the degrees of autoimmunity in Lyme disease warrant further investigation to distinguish pathogenetically related possibilities from epiphenomena.

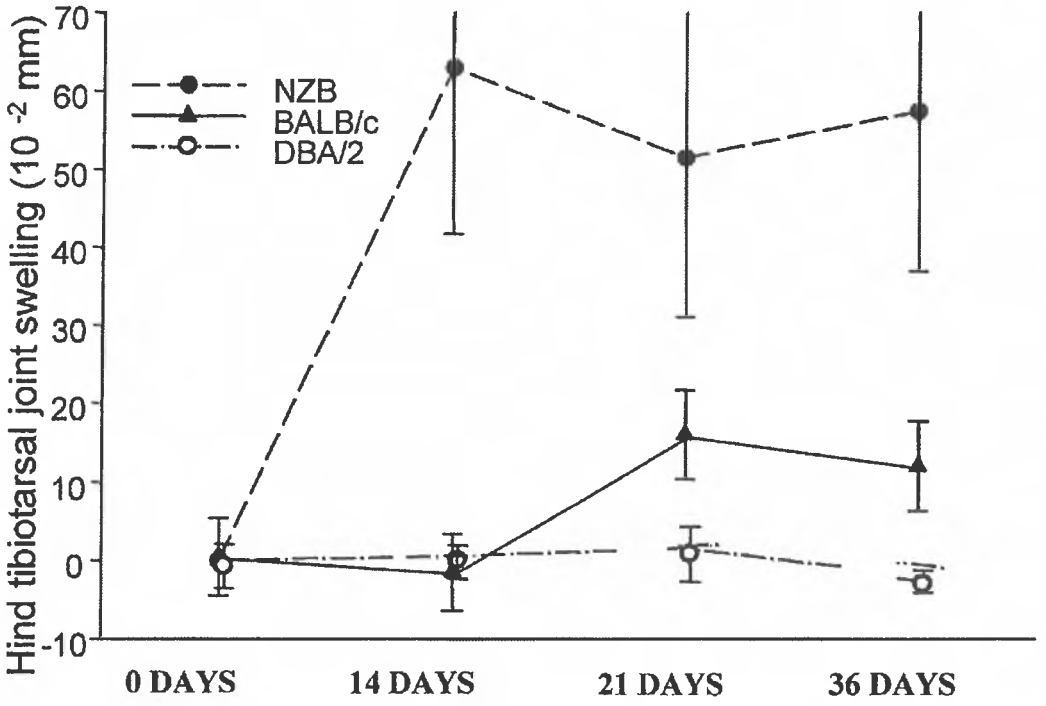

FIG. 1. Joint swelling in mice at various weeks after infection with *B. burgdorferi*. Mice were between 9 and 12 months of age at the time of initial infection. Data are means ± standard deviations (approximately five mice/group). Values are shown as the differences from uninfected time zero for each mouse. The NZB group was significantly different from the other groups on day 14 ($P < 0.05$; Student's *t* test).

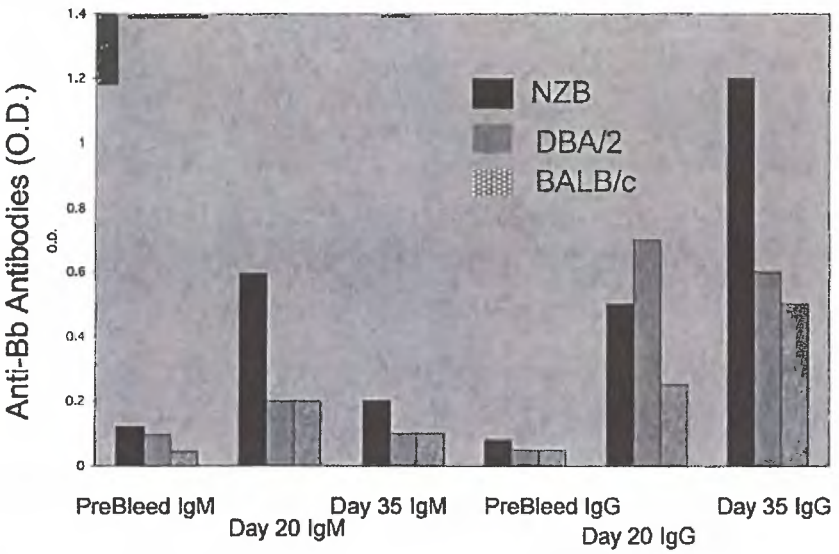

FIG. 2. Levels of anti-*B. burgdorferi* antibodies in *B. burgdorferi* (Bb)-infected animals at various times pre- and postinfection using equivalent serum dilutions. Data in columns represent mean values for OD (O.D.) readings in an ELISA of pooled sera from each group. Sera (diluted 1:10) were obtained from animals studied at 9 to 12

months of age. Columns are IgM (left three groups) and IgG (right three groups) antibodies reactive with *B. burgdorferi*.

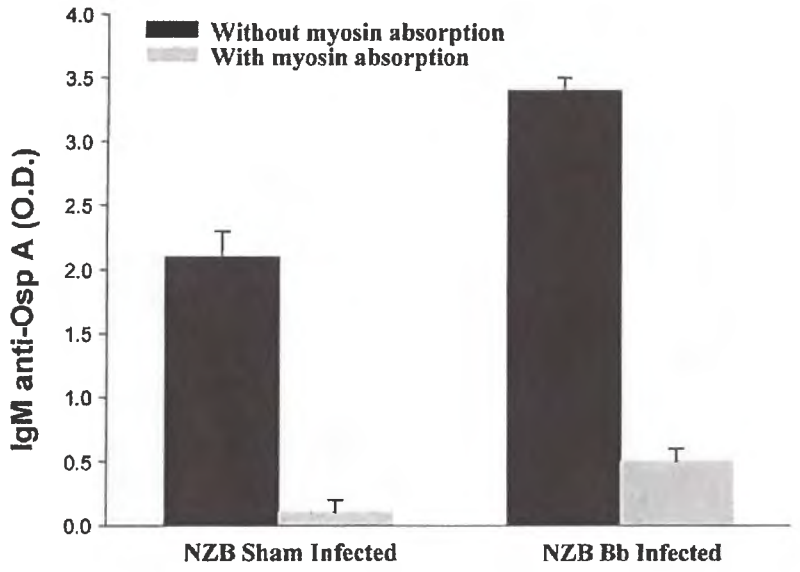

FIG. 3. Levels of IgM anti-OspA antibodies in control and *B. burgdorferi*-infected NZB mice infected between 5 and 6 months of age and bled on day 35 postinfection. Data in columns represent mean values for OD (O.D.) readings in an ELISA.

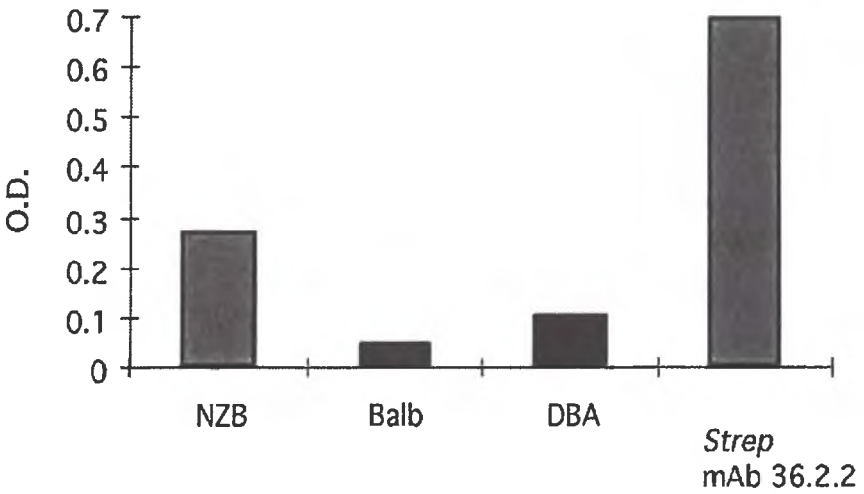

FIG. 4. The mean ELISA OD (O.D.) readings of IgM anti-myosin reactivity in pooled sera of groups of mice infected with *B. burgdorferi* 35 days prior to readings. The anti-*S. pyogenes* (*Strep*) M5 MAb 36.2.2 was employed for comparison.

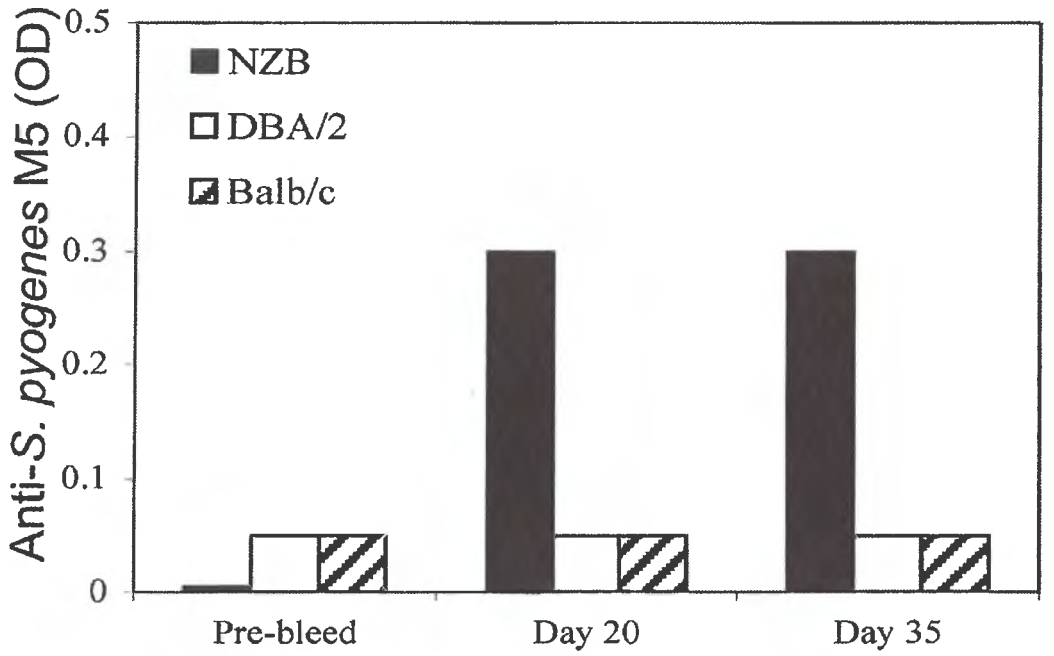

FIG. 5. Levels of IgM anti-*S. pyogenes* M5 antibodies in *B. burgdorferi*-infected animals at various times pre- and postinfection. Data in columns represent mean values for OD readings in an ELISA. Pooled sera (diluted 1:10) were obtained from animals studied at 9 to 12 months of age (approximately five mice/group).

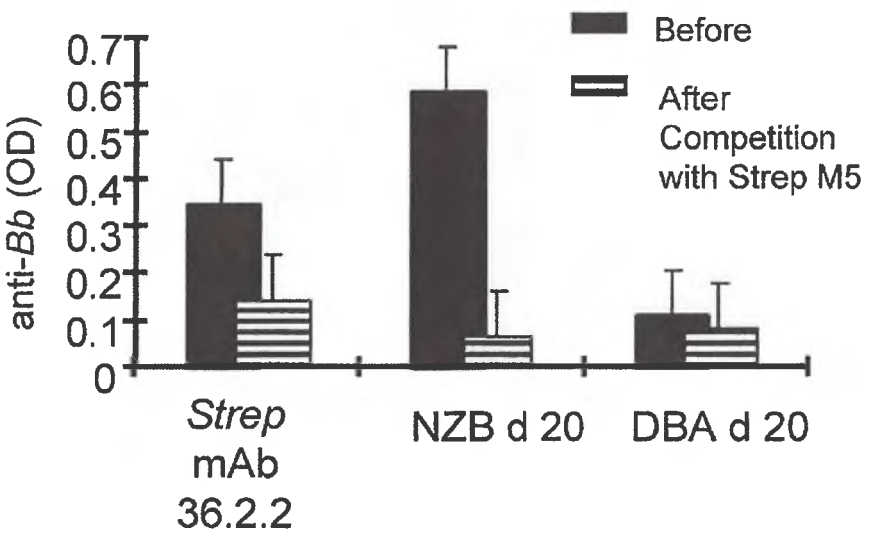

FIG. 6. Competition of anti-*B. burgdorferi* (anti-*Bb*) reactivity with *S. pyogenes* M5 protein. Sera from 9- to 12-month-old individual NZB and DBA mice obtained on day 20 postinfection with *B. burgdorferi* or culture supernatants from anti-*S. pyogenes* (*Strep*) MAb 36.2.2 were preincubated with 10 μg of *S. pyogenes* M5 protein and then tested by ELISA for reactivity with *B. burgdorferi* lysates. Data represent mean OD values.

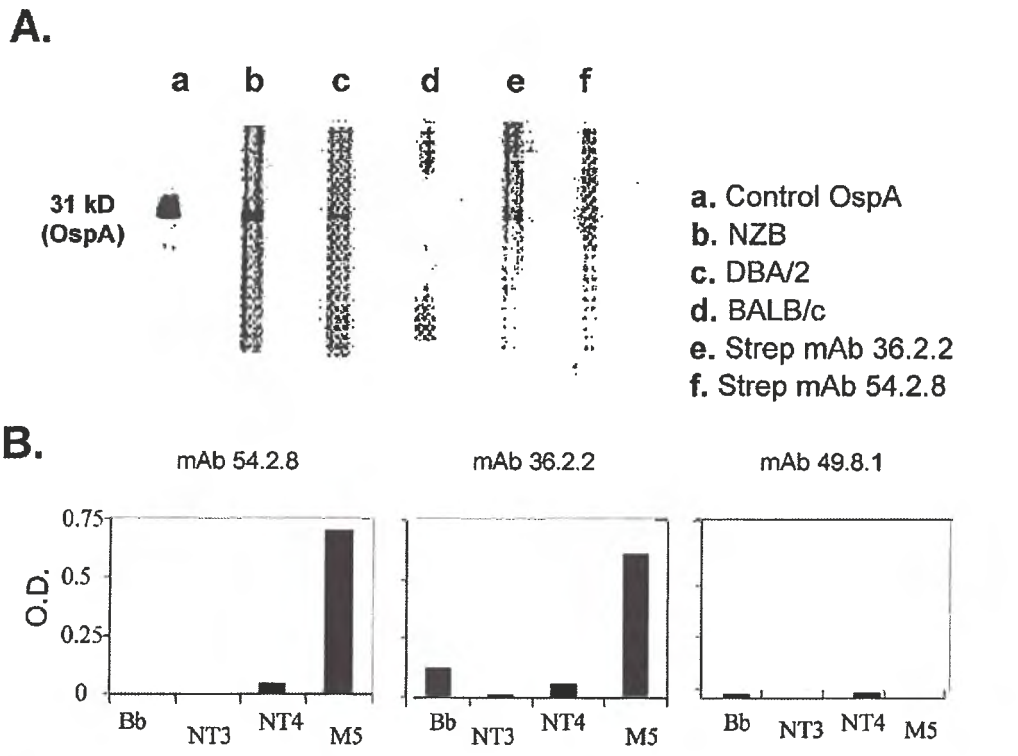

FIG. 7. (A) Western blot immunoreactivity of serum or monoclonal antibodies to purified recombinant OspA lipoprotein. The control lane contains anti-OspA monoclonal antibody which detects the 31-kDa OspA protein. Sera were obtained from *B. burgdorferi*-infected NZB (lane b), DBA/2 (lane c), and BALB/c (lane d) mice 35 days following infection. Also shown are anti-*S. pyogenes* (Strep) M5 IgM MAbs 36.2.2 (lane e) and 54.2.8 (lane f). (Anti-OspA reactivity was detected with specific conjugated goat anti-mouse IgM in all lanes except the control lane, which was reacted with goat anti-mouse IgG.) (B) Anti-*S. pyogenes* monoclonal antibodies were screened for binding to ELISA plates coated with either *B. burgdorferi* sonicates, purified M5 protein, or control subpeptides NT3 and NT4. Data in columns represent mean values for OD readings in an ELISA.

TABLE 1. Persistent presence of *B. burgdorferi* in the hearts of infected mice

Mice	Time of *B. burgdorferi* infection	% PCR positive for OspA (no. positive/total)[a]
NZB	None (control)	0 (0/3)
NZB	6 weeks	60 (3/5)
NZB	8 months	100 (4/4)

[a] Each group was tested for the OspA gene in the heart and spleen by semiquantitative PCR.

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Lyme borreliosis: from infection to autoimmunity

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ABSTRACT

Lyme borreliosis in humans is an inflammatory disease affecting multiple organ systems, including the nervous system, cardiovascular system, joints and muscles. The causative agent, the spirochaete *Borrelia burgdorferi*, is transmitted to the host by a tick bite. The pathogenesis of the disease in its early stages is associated largely with the presence of viable bacteria at the site of inflammation, whereas in the later stages of disease, autoimmune features seem to contribute significantly. In addition, it has been suggested that chronic persistence of *B. burgdorferi* in affected tissues is of pathogenic relevance. Long-term exposure of the host immune system to spirochaetes and/or borrelial compounds may induce chronic autoimmune disease. The study of bacterium–host interactions has revealed a variety of proinflammatory and also immunomodulatory–immunosuppressive features caused by the pathogen. Therapeutic strategies using antibiotics are generally successful, but chronic disease may require immunosuppressive treatment. Effective and safe vaccines using recombinant outer surface protein A have been developed, but have not been propagated because of fears that autoimmunity might be induced. Nevertheless, new insights into the modes of transmission of *B. burgdorferi* to the warm-blooded host have been generated by studying the action of these vaccines.

Keywords *Borrelia burgdorferi*, review, spirochaetal persistence, tick-borne disease, virulence

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INTRODUCTION

Lyme borreliosis is an inflammatory disease caused by the spirochaete *Borrelia burgdorferi*. This Gram-negative microorganism is transmitted to a variety of hosts by ticks, mainly of the genus *Ixodes*. Lyme borreliosis in humans manifests as a multisystem disorder of the skin and other organs, such as joints, cardiac system, nervous system and eyes.

Since surveillance for Lyme disease was begun by the Centers for Disease Control in 1982, the number of cases reported each year in the USA has increased dramatically to about 15 000, making Lyme disease the most common vector-borne disease in the USA [1]. In Europe, Lyme borreliosis has been documented widely in forested areas. The highest frequencies of the disease have been reported from Scandinavia, Germany, Austria, Slovenia and Sweden [2]. The infection has also been reported in Russia, China and Japan.

Ticks of the genus *Ixodes* undergo larval, nymphal and adult stages during their life cycle. The risk of infection in a given area depends largely on the environmental density of ticks, their feeding habits and their animal hosts. Small mammals, particularly rodents, are important hosts of ticks and are critical for maintenance of *B. burgdorferi* in nature. In addition, deer serve as hosts, especially during the adult tick stages [3,4]. The predominant tick species that transmit *B. burgdorferi* to humans are the Eurasian species *Ixodes ricinus* and *I. persulcatus*, and the North American species *I. dammini*, *I. pacificus* and *I. scapularis* [5]. The larval stage of the insect that emerges from the egg passes through a nymphal stage before developing into an adult.

The tick feeds only once at each stage in its life cycle. Activity of the ticks is seasonal. The tick bite is usually painless, and therefore may go unnoticed. Indeed, more than half of affected individuals do not remember a tick bite. During feeding, the tick may transmit *B. burgdorferi* through its

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saliva. *B. burgdorferi* is distributed in the mid-gut of the infected ticks. It is thought that, once the tick has its blood meal, *B. burgdorferi* penetrates the gut mucosa, disseminates into other tissues, including the salivary gland, and is inoculated into the host within 12–72 h [6].

In Europe, the proportion of ticks reported to harbour *B. burgdorferi* ranges from 0% to 85%; in the USA, it varies from 1% to 100%. This percentage depends on the developmental stage and the prevalence of infection of the ticks. Infection is highest in the adult and nymph forms of the tick, and lowest among the larval forms [7]. Ovarian transmission of *B. burgdorferi* from the mother tick to the offspring is possible [8]. *Borrelia* can invade the developing oocyte yolk complex in the ovary from the haemolymph before the impervious shell forms around the egg [9,10]. During embryonic development, spirochaetes can migrate from the yolk region to neuronal ganglia. Ovarian transmission in ticks can be very efficient, with passages over five to nine generations of ticks having been documented [10].

BIOLOGY OF *BORRELIA BURGDORFERI*

The aetiological agent *B. burgdorferi sensu lato* has been subdivided into three species causing human Lyme disease: *B. burgdorferi sensu strictu*, *B. afzelli* and *B. garinii*. Strains of all three species have been isolated from patients in Europe, whereas only the first species has been reported in the USA.

Borrelia spp. vary in length, diameter, tightness of the coils, and number of periplasmic flagella. The length can range from 10 to 30 μm and the width of the helices from 0.2 to 0.25 μm [11]. The generation time under optimal conditions (30–40°C, microaerophilic) is 7–20 h. Ultrastructurally, the causative agent resembles other spirochaetes of the genus *Borrelia*, with a non-patterned surface layer, a three-layered outer-membrane surrounding a periplasmic space containing the variable number of flagella, and the protoplasmic cylinder [12] (Fig. 1).

B. burgdorferi has a linear chromosome in addition to linear and circular plasmids. The genes that encode major outer-surface proteins (Osps) are located on plasmids [13]. *B. burgdorferi* strains usually have three major Osps (OspA, OspB, OspC) [13], along with OspD [14], OspE, OspF [15] and OspG.

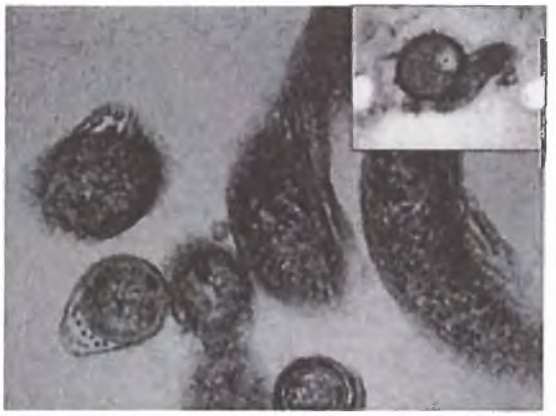

Fig. 1. Transmission electronmicrograph of *Borrelia burgdorferi* strain *sensu strictu* showing the corkscrew shape and motility, and the outer-membrane surrounding a periplasmic space that contains the variable number of flagella and the protoplasmic cylinder. Insert: Immuno-electronmicrograph using a polyclonal antiserum against *Borrelia burgdorferi*, demonstrating that a bleb formed during in-vitro culture is an integral part of the bacterial cell.

The loose association of the outer-envelope with the underlying protoplasmic cylinder leads to the separation of the cell components when borrelia are kept in hypotonic solution [16]. This so-called bleb formation has been observed on the outer-envelope of *Borrelia in vitro* when a specific antibody or complement is added [17] (Fig. 1). Studies have indicated that exposure to physiological stress, e.g., changes in pH, depletion of metabolites and ageing [17], or exposure to antibiotics [18,19], can lead to bleb formation. Little is known about the physiological role of these forms, although blebs and gemmae have been shown to contain DNA [20] and may be involved in the exchange of genetic information.

Alban *et al.* [21] reported that serum starvation resulted in the transformation of motile helical forms into non-motile, spherical cysts containing tightly coiled spirochaetes. Addition of tetracycline inhibited the formation of cysts, demonstrating that cyst formation requires protein synthesis. Unlike cyst forms, blebs and gemmae have not been shown to be viable, and seem to be incapable of transforming back into motile vegetative bacteria [21]. Cysts have been observed in human cerebrospinal fluid (CSF) [22] and in the tissues of patients infected with Lyme disease [23,24]. Thus, if viable cysts can form in the human body, they may represent a strategy that facilitates the survival of *B. burgdorferi* during

nutritionally adverse conditions in host tissues. By forming cysts, it is also conceivable that *B. burgdorferi* might evade detection by the host immune system [21]. Hulinska *et al.* [24] demonstrated that the surface of cysts found in erythema migrans of infected human tissue was non-reactive to antibodies against OspA, whereas the content of the cysts did react with OspA [24].

Periplasmic flagella can be found beneath the outer-membrane, and these mediate spirochaete motility. Borrelial flagella resemble one another and basically have the same structural organisation as the flagella of other eubacteria [25]. Like other spirochaete flagella, borrelial flagella also insert sub-terminally and with bipolarity. Different *Borrelia* spp. have different numbers of flagella [26]. The flagella have four components—the filament, the hook, the neck and the basal disk [27]—and resemble flagella of Gram-negative bacteria [28]. While treponemal flagella are sheathed, borrelial flagella are characteristically unsheathed [29] and are composed predominantly of flagellin protein with a molecular mass of 41 kDa [30].

The borrelial cell membrane contains muramic acid [31] and ornithine as a diamino acid in a peptidoglycan structure [32]. A unique feature of *Borrelia* spp. is that the genes for the immunodominant outer-surface proteins OspA, OspB and OspC are located on plasmids. Although the outer-surface proteins are immunologically and genetically variable [33], OspA [34] and OspC [35] have proven to be very useful proteins for the development of a *Borrelia* vaccine. Other important antigens of *B. burgdorferi* are the flagellin protein and a 60-kDa antigen. The latter has been termed a common antigen and belongs to the heat-shock protein family. The flagellin protein and the 60-kDa antigen are not species-specific.

The complete genome of *B. burgdorferi sensu strictu* (strain B31) has now been sequenced [3]. The genome is quite small (*c.* 1.5 Mb) and consists of a highly unusual linear chromosome of 950 kb, together with nine linear and 12 circular plasmids. The outer-surface proteins of *B. burgdorferi* presumably help the spirochaete to adapt to and survive in markedly different environments between cold-blooded ticks and mammals [4].

B. burgdorferi undergoes dramatic metamorphosis, including changes in expression of many different lipoproteins, when it transfers from the tick to the mammalian environment. Inside the mid-gut of unfed ticks, *B. burgdorferi* expresses the OspA lipoprotein in large amounts [36]. Immunofluorescence studies have shown that after the tick attaches to the mammalian host and begins feeding, *B. burgdorferi* starts to down-regulate OspA expression, and rapid synthesis of OspC begins [37]. At the same time, *B. burgdorferi* migrates from the tick mid-gut to the salivary glands in preparation for mammalian host infection. Once *B. burgdorferi* is inside the mammalian host, the transition from OspA to OspC expression is complete. Now *B. burgdorferi* no longer expresses OspA, while OspC is detectable readily on spirochaetes that have adapted to the mammalian host [38].

Temperature adaptation is one important factor that *B. burgdorferi* uses to undergo this transition. Changes in lipoprotein expression can also be demonstrated by comparing *B. burgdorferi* grown in culture at 23–24°C with that grown at 35°C [37]. The effects of temperature on the differential expression of OspA and OspC are enhanced by co-cultivation of *B. burgdorferi* with tick cells [39]. In addition to OspC, other lipoproteins are also up-regulated during the transition to the warm-blooded mammalian host. Thus, when culture conditions are changed from low to high temperatures, up-regulated expression of the two outer-surface proteins OspE and OspF can be observed [40]. It has become evident that spirochaetal proteins, up-regulated during infection of mammals, are members of a large family of OspE- and OspF-related proteins, currently designated Erps [41]. Further studies have shown that expression of Erp proteins can be induced in the cultivated *B. burgdorferi* strain B31 by a shift in temperature from 23°C to 35°C [40].

Incubation of spirochaetes at different temperatures is one method of mimicking environmental signals that can regulate lipoprotein expression. In addition, another surface-exposed lipoprotein has been reported to undergo extensive antigenic variation during early disseminated infection [42]. The genome does not contain any homologue for the system that enables the organism to secrete toxins and other virulence factors. So far, the only known virulence factors of *B. burgdorferi* are surface proteins that allow the spirochaete to attach to mammalian cells. The organism produces few proteins with biosynthetic activity, and apparently depends on the host for many of its nutritional requirements.

CLINICAL FEATURES OF LYME DISEASE

Lyme borreliosis in adults can be divided into three clinical stages [43–45] that may overlap with each other. The first two stages, which appear within a few weeks or months after infection with *B. burgdorferi*, represent the early phase of the disease. The third, or late, phase appears after several months or years [46]. The early features of the disease are usually self-limiting, but late features may become chronic and progressive. Previous exposure to *B. burgdorferi* does not prevent infection; indeed, the occurrence of erythema migrans in a patient with acrodermatitis chronica atrophicans has been reported [44].

Stage I: Early infection

In the early stage of infection, erythema migrans develops regularly, but can be absent in up to 50% of patients (for details of the clinical morphology, see [45]). The skin lesion is frequently accompanied by influenza-like symptoms, such as malaise, fatigue, headache, fever and regional lymphadenopathy. In Europe, erythema migrans often remains an indolent localised infection, whereas in the USA this lesion has been associated with more intense inflammation and signs that often suggest early dissemination of the spirochaete [47]. In one study, spirochaetes could be cultured from plasma samples in 50% of patients affected with erythema migrans [48]. The lymphocytoma, another acute skin lesion, appears typically on the nipple or areola of the breast [49], or on the ear lobes [45]. These lesions are usually caused by *B. afzelli* or *B. garinii* [50], and are therefore reported mainly in Europe, but not in the USA. A few weeks to months after infection, several organs may become affected, probably because of haematogenous spread of the pathogen.

Stage II: Early dissemination

After a tick bite, *B. burgdorferi* might spread from the site of the bite into the bloodstream, causing clinical signs of early dissemination. Lyme disease can affect the nervous system. Neural manifestations during this stage are meningoradiculoneuritis (Bannwarth's syndrome), meningitis, plexus neuritis, cranial neuritis (predominantly involving facial nerves) and mononeuritis multiplex. Bannwarth's syndrome, which in Europe is the most common neurological manifestation in adults, is characterised by CSF lymphocyte pleocytosis and intense radicular pain. The spread of *B. burgdorferi* within the nervous system has been demonstrated in non-human primates [51], the only known model of neuroborreliosis. In immunocompromised monkeys, which have a larger spirochaetal burden than immunocompetent animals, *B. burgdorferi* has been shown to infiltrate leptomeninges, sensory and motor nerve roots, and the dorsal root ganglion, but not the brain parenchyma [52]. In the peripheral nervous system, spirochaetes have been detected in the perineurium, the connective tissue sheath surrounding each bundle of peripheral nerve fibres.

Few patients suffering from Lyme disease develop heart problems. Most commonly, atrioventricular blockage has been described, and occasionally acute myopericarditis or mild left ventricular dysfunction, but rarely cardiomegaly [43]. These heart abnormalities can appear several weeks after infection. In Europe, *B. burgdorferi* has been isolated from endomyocardial biopsy samples from several patients with chronic dilated cardiomyopathy [53].

Arthralgia and myalgia indicate early musculoskeletal involvement. Frank arthritis and myositis can also be observed occasionally in the first few months of the disease. Regional lymphadenopathy and generalised lymphadenopathy may develop.

Stage III: Chronic disease

Chronic organ involvement may develop years after the tick bite. Skin and soft tissue manifestations are common in Lyme borreliosis [45]. Acrodermatitis chronica atrophicans starts as an inflammatory dermatitis. Later, this lesion evolves into an atrophic skin lesion.

Among untreated patients in the USA, *c.* 60% begin to have intermittent attacks of joint swelling and pain. Large joints, especially the knees, are primarily affected [54]. Synovial tissue from affected patients shows synovial hypertrophy, vascular proliferation and marked infiltration of mononuclear cells. Furthermore, patients with Lyme borreliosis usually have higher *Borrelia*-specific antibody titres in serum than do patients with any other manifestation of the disease. After several short attacks of arthritis, some patients may develop persistent joint inflammation.

Direct involvement of the eye (keratitis, optic neuritis) has also been attributed to *B. burgdorferi* infection [43,55]. However, since *B. burgdorferi* has been isolated rarely from patients with these opthalmological disorders, the pathogenesis in these cases is uncertain.

In both the USA and Europe, a chronic axonal polyneuropathy may develop, manifested primarily as spinal radicular pain [56,57]. Electromyograms typically show diffuse involvement of proximal and distal nerve segments. In Europe, *B. garinii* may cause chronic encephalomyelitis, characterised by cranial neuropathy with marked intrathecal production of antibodies against the spirochaetes [56]. In the USA, a mild late neurological syndrome has been reported, termed Lyme encephalopathy. It is characterised by memory deficit, minor depression, irritability and somnolence [58–61].

The differences between genospecies found in Europe and North America may account for differences in the frequencies of certain manifestations of Lyme disease in these areas. For example, neurobiological manifestations of Lyme disease are more common in Europe, whereas rheumatological manifestations are more common in North America.

Perinatal Lyme disease

Case reports have suggested that adverse outcomes of pregnancies may be complicated by maternal Lyme borreliosis [62]. The risk of transplacental transmission of *B. burgdorferi* is probably minimal when appropriate antibiotics are given to a pregnant woman with Lyme borreliosis. There have been several published case series investigating the relationship between gestational Lyme disease and fetal outcome. The questions addressed in these series were as follows. Does *B. burgdorferi* cross the placenta and invade the foetus? If there is transplacental transmission, does this have any significance for the development of the foetus? Several studies have shown a relationship between seropositivity in pregnancy and pregnancy outcome. A large serological study of 2014 women, of whom 12 were seropositive, revealed no increased risk of congenital malformations, low birth weight, abnormal length of gestation or risk of fetal death among children born to seropositive mothers [63]. A second study of 1416 pregnant women, of whom 12 were seropositive at delivery, also revealed no adverse outcomes attributable to seropositivity [64]. A study comparing 5000 infants, divided equally between a Lyme-endemic area and a control area, showed no significant differences in the incidence of congenital malformations, except for a statistically significant increase in the rate of cardiac malformations in the Lyme-endemic area [65]. However, it is not known whether this finding represents an artefact or a valid difference between the two populations. A further epidemiological study conducted in a Lyme-endemic area has questioned the connection between maternal Lyme disease and congenital heart disease [66]. In this study of 796 patients and 704 control subjects, there was no significant association between congenital defects and maternal Lyme disease. In another clinical study, a higher incidence of neurological disorders was not found in children of women with gestational Lyme disease in an epidemic area.

Studies in both human and animal models have established that *B. burgdorferi* can cross the placenta, presumably during the period of initial spirochaetaemia. Despite documentation of transplacental transmission of *B. burgdorferi*, there has been no clinical evidence for a fetal inflammatory or immune response, or an adverse neonatal outcome resulting from gestational Lyme disease [67]. The above studies indicated that an adverse fetal outcome resulting from maternal infection with *B. burgdorferi* at any point during pregnancy in humans is, at most, extremely rare.

Lyme disease in children

In principle, manifestations among children are the same as in adults. However, Lyme disease in children follows a somewhat different course and has different symptoms than in adults (Table 1). In most cases, the clinical and epidemiological features are comparable among children suffering from Lyme disease in Europe and the USA [68,69]. Children account for a relatively high number of Lyme borreliosis patients, presumably because of greater exposure to ticks and decreased attention to prevention of Lyme disease. Frequently, tick bites in children occur on the upper parts of the body, and especially on the head. Thus, modes of pathogen dissemination inside the host, especially into the central nervous system, might occur faster than in adults. This is reflected by the fact that

Table 1. Comparison of Lyme disease manifestations in children and adults

Course of disease	Children	Adults
Early manifestations (days to a few weeks)		
General symptoms	Influenza-like disease	Influenza-like disease, lymphadenopathy
Skin	Erythema migrans, lymphocytoma	Erythema migrans, lymphocytoma
Neurological	Lymphocytic meningitis Cranial neuritis, mainly the facial nerve	
Heart	Myopericarditis	
Eye	Conjunctivitis	
Joint; muscle	Arthralgias	
Early dissemination (after a few weeks)		
General symptoms		Lymphadenopathy
Neurological		Meningitis Meningoradiculoneuritis (Bannwarth's syndrome) Plexus neuritis, cranial neuritis Mononeuritis multiplex
Heart		Atrioventricular blockage Myopericarditis, cardiomyopathy
Joint; muscle		Arthralgia, myalgia, oligoarthritis
Late stage of infection, chronic disease (months to years after infection)		
Skin	Acrodermatitis chronica atrophicans (rare)	Acrodermatitis chronica atrophicans
Neurological	Meningoradiculoneuritis (rare) Encephalomyelitis (rare)	Axonal, sensory polyneuropathy Cranial neuropathy Chronic encephalomyelitis, encephalopathy Cardiomyopathy (rare)
	Heart	Cardiomyopathy
Eye	Uveitis, keratitis	Retinitis, uvetis, keratitis, endophthalmitis
Joint; muscle	Episodic or chronic oligoarthritis	Treatment-resistant arthritis

meningitis in children occurs frequently early in the course of the disease, and often in parallel with erythema migrans. Lymphocytic meningitis, with or without cranial neuropathy, is the major early neurological manifestation of Lyme disease in children, and generally presents with episodic headache, mild neck stiffness, numbness and poor motor co-ordination.

Eppes *et al.* [70] compared Lyme meningitis with viral meningitis in children. Like Lyme meningitis, viral meningitis occurs during summer, and is difficult to diagnose in Lyme-endemic regions, but it was suggested that some clinical and laboratory findings in Lyme meningitis are sufficiently distinctive: (1) cranial neuropathy, especially peripheral seventh nerve palsy, is strong evidence of Lyme meningitis; (2) papilloedema is more likely to be seen in Lyme meningitis than in viral meningitis; (3) a longer duration of symptoms before hospital admission supports a diagnosis of Lyme meningitis; (4) fever at the time of diagnosis is more likely to be related to viral meningitis; and (5) CSF pleocytosis is less pronounced in Lyme meningitis than in viral meningitis, and especially the initial neutrophilic component [70]. Chronic late stage neurological disease, e.g., meningoradiculoneuritis or encephalomyelitis, is rare and is not as frequent as joint disease [62].

Lyme borreliosis may be the cause of a variety of inflammatory changes of the eye occurring in childhood, either early (conjunctivitis) or late (uveitis, keratitis) [55]. In a German study of 84 children with arthritis caused by late Lyme borreliosis, three had borrelial eye disease, including keratitis, anterior uveitis and uveitis intermedia [71]. Clinicians should be aware that optic nerve involvement may be a manifestation of Lyme disease, because of either inflammation or increased intracranial pressure, or both [55]. Among patients with symptoms suggestive of Lyme disease, decreased vision suggests the possibility of optic neuritis, whereas the presence of headache, visual symptoms, pulsatile tinnitus, sixth nerve palsy or papilloedema can be important signs of increased intracranial pressure. A few children in the USA have been reported with optic neuritis associated with Lyme disease, which occurred 1–9 months after initial infection [55,72–75].

Younger children (aged < 10 years) are more likely to have fever at onset of arthritis, followed by an acute or episodic course, and to have lower antibody titres to *B. burgdorferi* compared to adults [76,77]. It has been documented that the clinical characteristics of children with Lyme arthritis vary with age. Among adults with Lyme arthritis,

c. 10% are reported to develop chronic arthritis. In children with Lyme arthritis, a chronic course of arthritis seems less frequent [78,79]; however, in a European study, 24% of children affected by Lyme arthritis retained manifestations of disease, including arthritis and arthralgias, 12 months after antibiotic treatment [76]. In contrast to adult patients, temporomandibular or sternoclavicular joint involvement is rare in children.

PATHOGENESIS

Pathogenic factors encoded by spirochaetes

Most of the sub-surface lipoproteins of *B. burgdorferi* play an important role in cellular physiology and participate directly in pathogenesis [3,80]. Mutants deficient in OspA are more sensitive to complement lysis and digestion by proteases in the mid-gut of ticks [81,82]. These facts suggest that OspA expression by *B. burgdorferi* during tick infection might protect the organism both from tick mid-gut proteases and from mammalian complement when the tick takes blood from the host. The ability of spirochaetes to attach to eukaryotic cell surfaces and extracellular matrix proteins is also essential in pathogenesis.

Several pathogenic mechanisms may aid in the dissemination of *B. burgdorferi*. For example, the sequences of OspC differ considerably among *Borrelia* strains, and only a few particular sequences have been associated with dissemination of disease [83]. Spread through skin and other tissue matrices may be facilitated by the binding of human plasminogen and its activators to the surface of the spirochaete [84]. During the dissemination and homing of *B. burgdorferi* to specific sites, the pathogen attaches to certain host integrins [85], matrix glycosaminoglycans [86] and extracellular matrix proteins [87].

Decorin is a collagen-associated glycosaminoglycan found in various tissues, including skin and joints, i.e., sites typically associated with Lyme disease. *B. burgdorferi* has been shown to attach selectively to decorin, which led to the subsequent isolation of decorin-binding protein (Dbp) genes by screening an expression library with digoxigenin-labelled decorin [86,88]. The two gene operons encoding the lipoprotein DbpA and the related DbpB were identified independently by screening a *B. burgdorferi* expression library [89,90] and by sequencing DbpA found in *B. burgdorferi* outer-membranes [91]. *Borrelia* decorin-binding proteins A and B bind decorin on collagen fibrils, which may explain why the organism is commonly aligned with collagen fibrils in the extracellular matrix of the heart, nervous system and joints. In a recent report, decorin-deficient mice had more limited spirochaetal colonisation of joints, and milder arthritis, than normal mice of the same strain that expressed decorin [92]. DbpA demonstrates considerable heterogeneity among *B. burgdorferi* strains. Chemical modification of lysine residues was found to abrogate DbpA binding to decorin.

Involvement of the infected host

Several studies have suggested that *B. burgdorferi* is present at the site of inflammation in many clinical manifestations of Lyme disease. *B. burgdorferi* does not produce toxins, but is a potent immunomodulator. The effects of *B. burgdorferi* on local cells may lead to suppression of the local immune response. A decrease in the expression of major histocompatibility complex (MHC) markers on Langerhans' cells in the skin of patients with acrodermatitis chronica atrophicans has been demonstrated after contact with *Borrelia* [93].

B. burgdorferi infection has been shown to increase the expression of neural cell adhesion molecule on endothelial cells in comparison with controls [94]. After *B. burgdorferi* has entered the skin, it can move through the extracellular matrix. *B. burgdorferi* can bind to the components of the extracellular matrix, including epithelial cell-derived proteoglycans, by interacting with decorin [88], glycosaminoglycans [95] and fibronectin [96,97]. Many studies have shown that *B. burgdorferi* can attach to human umbilical vein endothelial cells (HUVECs) *in vitro*, and that it can traverse HUVEC monolayers grown on tissue substrate. This penetration may be either between or through the HUVECs [98]. HUVECs exposed to *B. burgdorferi* in in-vitro culture show increased expression of E-selectin, vascular cell adhesion molecule and intercellular cell adhesion molecule.

Other resident joint and vascular endothelial cells seem to react differently. Synovial fibroblasts have been shown to down-regulate intercellular cell adhesion molecule-1 after contact with *B. burgdorferi*. Vascular cell adhesion molecule expression was not changed in these cells after

infection with *B. burgdorferi*. In addition, the expression of nitric oxide synthase was not altered by the infection [99]. It is of particular interest that synoviocytes strongly up-regulate these molecules during an inflammatory response. Thus, these local immunomodulatory features might be sequelae of *B. burgdorferi* residing, or even persisting, in the cytosol of resident joint cells, as has been demonstrated *in vitro* (Fig. 2) [100]. However, intracellular persistence of a spirochaete has not so far been demonstrated consistently *in vivo*.

Despite robust humoral and cellular immunity against *B. burgdorferi* in most patients, the disease can become chronic, even after several courses of antibiotic treatment. The pathogenesis of chronic Lyme disease remains a topic of discussion, currently focusing on the concepts of persistent infection and/or autoimmunity. Recently, chronic joint inflammation has been attributed to autoimmunity [101,102].

There is strong evidence that T-lymphocytes play a major role in the pathogenesis of Lyme arthritis. It has been shown that the proliferative response of *B. burgdorferi*-specific T-lymphocytes isolated from the synovial fluid or peripheral blood of adults [103–107] and children with Lyme arthritis [108,109] is elevated. In addition, T-lymphocytes from individuals with prolonged Lyme arthritis responded more vigorously to stimulation with *B. burgdorferi* antigens than the T-lymphocytes from adult individuals affected by less progressive forms of Lyme arthritis [110,111]. Likewise, cloned T-lymphocytes from a patient with chronic Lyme arthritis exhibited enhanced proliferative responses to various *B. burgdorferi* antigens [112].

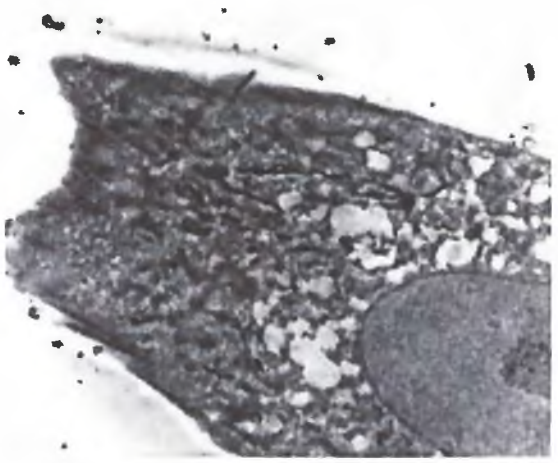

Fig. 2. Transmission electronmicrograph of a human synovial cell infected with *Borrelia burgdorferi in vitro*, demonstrating an intracellular cytosolic location of the spirochaetes, identified by multiple corkscrew-shaped structures in the cytosol.

Many investigators have demonstrated that individuals with progressive forms of Lyme arthritis frequently have T-lymphocytes that liberate lymphokines characteristic of the Th1 phenotype [112,113]. The existence of highly polarised Th1 lymphokine patterns has also been described in mice infected with *B. burgdorferi*. Other studies [114–116] have shown a relationship between T-cell phenotypes and the induction or control of Lyme arthritis. T-cell involvement in the pathogenesis of Lyme arthritis has been described in a hamster model of Lyme borreliosis. Lymph node T-cells from hamsters vaccinated with 10^8 formaldehyde-inactivated spirochaetes in adjuvant are able to confer susceptibility to severe Lyme arthritis when transferred into naive hamsters challenged with 10^6 viable *B. burgdorferi* cells [114]. When primed T-cells were infused into naive recipients and challenged with dead *B. burgdorferi*, no arthritis was seen [114].

These studies suggest that gene products expressed actively by the spirochaete inside a mammalian host might be involved in the propagation of Lyme arthritis mediated by T-cells [117]. Hence, T-cells seem to be responsible for the development of destructive Lyme arthritis [118]. However, protection from disease by $CD8^+$ T-cells has been reported [116].

In the initial phases of disease, humoral immune responses in general are limited, or at least specific antibodies are hard to detect [119]. B-cell reaction to the pathogen might be abrogated by early antibiotic treatment [120]. Therefore, serological testing after early antibiotic treatment of erythema migrans is mostly unremarkable and is not recommended [121]. In the chronic phases of disease, a robust B-cell response against a variety of *Borrelia* epitopes is generally detectable [122] and can be used for serodiagnosis. However, this humoral response seems not to be protective, since it cannot prevent reinfection of the host with *B. burgdorferi*. Children and adults do not differ in their antibody response against *B. burgdorferi* [123]. The role of the B-cell response and antibody production in the pathogenesis of chronic Lyme disease is still to be elucidated.

It is well-established that T-lymphocytes of the Th1 phenotype are involved in the activation of

macrophages [124]. Macrophage activation may be central to the induction of Lyme arthritis [125,126]. Elevated levels of various macrophage-derived molecules (interleukin-1, tumour necrosis factor, prostaglandin E2 and collagenase) have been detected in synovial fluid extracts and serum from individuals with Lyme arthritis [127–130]. Interleukin-1 and tumour necrosis factor are known to activate osteoclastic cartilage and bone reabsorption, and may be responsible for the clinical and pathological manifestations of Lyme arthritis [131]. Macrophages can also be stimulated with *B. burgdorferi* to produce nitric oxide [132]. Production of nitric oxide has been associated with the induction of other arthritic diseases [133,134].

Autoimmune features of Lyme disease

One potential explanation for antibiotic-resistant Lyme disease is the generation of autoimmunity directly or indirectly mediated by the pathogen and based on molecular mimicry. Gajdusek [135] has suggested that axonopathy in a variety of neurological diseases might result from anti-axonal antibody production, and anti-axonal IgM antibodies have been demonstrated in the serum of patients with neurological Lyme disease [136]. Genetic linkage studies in adults with Lyme arthritis have demonstrated a link with MHC class II molecules DR2 and DR4 [137]. In addition, these patients develop anti-OspA antibodies correlating with the duration of their arthritis [138], suggesting that OspA may be involved in the autoimmune process. Self-reactive T-cells may maintain local inflammation [139].

MHC class II molecules play a critical role in the activation of the immune system. Polymorphisms within the genes encompassing the MHC class II structure influence the immune system by at least two mechanisms. First, polymorphic amino-acid residues on distinct class II proteins determine whether or not an individual peptide will bind and therefore be presented by a particular class II molecule displayed on an antigen-presenting cell. Second, MHC class II molecules regulate the developmental selection of T-cell receptor specificity in the thymus, thereby affecting the repertoire of $CD8^+$ and $CD4^+$ T-cells that recognise foreign peptides in the context of MHC class II molecules [117,140].

The first indication that treatment-resistant Lyme borreliosis might be an autoimmune disease came from a study analysing MHC II alleles (HLA-DR4) in patients with Lyme arthritis of brief, moderate or chronic duration [141]. Patients with chronic treatment-resistant Lyme arthritis have been found to have MHC II alleles that are also associated with rheumatoid arthritis, particularly HLA-DRB1* 0401 and 0101 alleles [142]. Most macrophages are able to internalise *B. burgdorferi* [143–147], and many investigators have demonstrated the localisation of intracellular *B. burgdorferi* within well-defined phagolysosomes [146,148,149] (Fig. 3). Filgueira *et al.* [150] showed that borrelial antigens were co-localised with MHC class II molecules within lysosomal vesicles of dendritic cells. These studies suggest that *Borrelia* antigens are loaded on to MHC class II molecules and are presented subsequently to $CD4^+$ T-lymphocytes.

It is of interest that synovial fibroblasts, which harbour *B. burgdorferi in vitro*, are able to present not only MHC I but also MHC II molecules [99,100]. Gross *et al.* [101] suggested that LFA-1 can serve as a cross-reactive autoantigen for OspA-reactive Th1 cells, leading to treatment-resistant Lyme arthritis. This was the first time that criteria for an autoimmune disease caused by molecular mimicry of microbial epitopes were

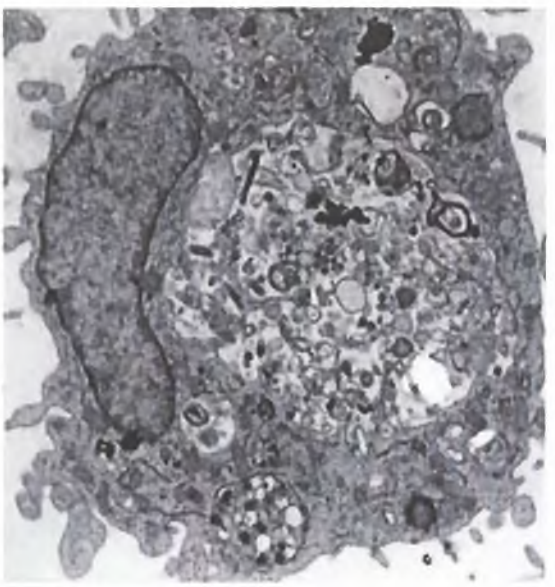

Fig. 3. Transmission electronmicrograph of a human peripheral blood monocyte, showing conventional phagocytosis of a *Borrelia burgdorferi* strain *sensu strictu* in a large central and smaller phagolysosome at the bottom of the figure.

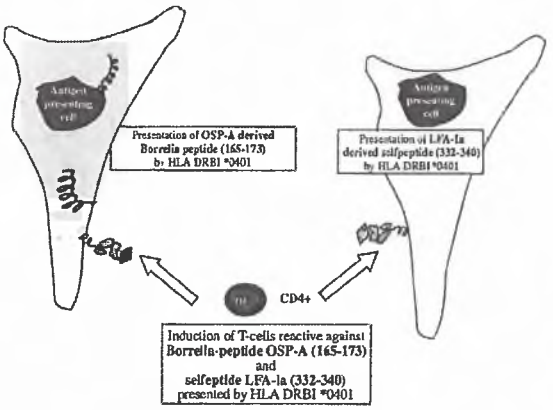

Fig. 4. Molecular mimicry in Lyme arthritis, depicting antigen-presenting cells (in this case monocytes, macrophages, dendritic cells and synovial fibroblasts) presenting peptides generated from borrelial OspA and host LFA-Ia (human leucocyte function-associated antigen 1), which induce a cross-reactive T-cell response.

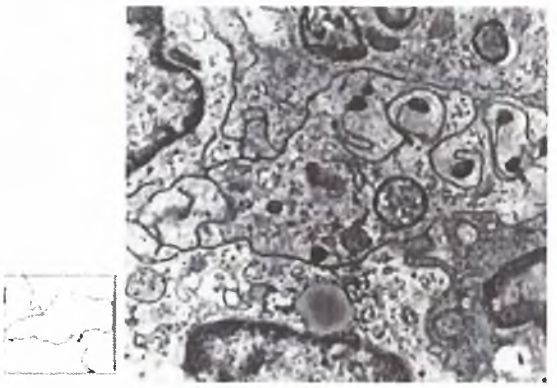

Fig. 5. Coiling phagocytosis of *Borrelia burgdorferi* by a cluster of human peripheral blood monocytes with their borders outlined in the scheme. The central cell shows multiple coiling phagocytosis figures probably trying to engulf one single spirochaete.

demonstrated in treatment-resistant Lyme arthritis [101,102,151–153]. Fig. 4 summarises these findings schematically, and suggests the possibility that intracellular persistence of *B. burgdorferi* in synovial cells might contribute to this scenario of molecular mimicry.

These studies were supported by the analysis of molecular mimicry in chronic neuroborreliosis. Hemmer *et al.* [154] demonstrated elegantly that several T-cell clones were responding to *Borrelia* peptides and endogenous host peptides. So far, these data on autoimmunity have been generated from adult Lyme disease patients. Comparable data on the pathogenesis of Lyme disease in children are pending.

Coiling phagocytosis has also been described as a mechanism for internalisation of *B. burgdorferi* [150,155–157] (Fig. 5). Coiling phagocytosis results in cytosolic deposition of antigens [150,156] and has been suggested as a mechanism for MHC class I presentation of exogenous antigens [158]. The presence of cytosolic *B. burgdorferi* has also been documented within human macrophages and granulocytes [156]. Likewise, an intracellular location of *B. burgdorferi* has been described for human dendritic cells [150], fibroblasts and synovial cells (Fig. 2) [100,159], endothelial cells [160] and murine marophages [146]. The ability of spirochaetes to enter the cytoplasm of macrophages may result in MHC class I presentation of *Borrelia* antigens to $CD4^-$ $CD8^+$ T-lymphocytes. In support of this, Busch *et al.* [116] demonstrated the existence of *B. burgdorferi*-specific $CD8^+$ T-lymphocytes in patients with Lyme borreliosis in remission. These studies suggest that *B. burgdorferi*-specific $CD8^+$ and $CD4^+$ T-lymphocytes can participate in the pathogenesis of Lyme arthritis.

DIAGNOSIS OF LYME DISEASE

The diagnosis of Lyme disease is based usually on recognition of the characteristic clinical features and a history of exposure in an area where the disease is endemic. For the diagnosis of an infectious disease, the standard is isolation of the causative agent in culture. Culture of *B. burgdorferi* from different specimens in Barbour–Stoenner–Kelly medium would permit a definitive diagnosis. However, positive cultures have generally only been obtained early in the course of disease, primarily from biopsy samples of erythema migrans lesions, less often from plasma samples [48], and only occasionally from CSF of patients with acute meningitis or facial paralysis [161]. At a later stage of the infection, PCR testing is greatly superior to culture for detection of *B. burgdorferi* in synovial fluid [162]. *B. burgdorferi* has not been isolated from the CSF of patients with chronic neuroborreliosis, but *B. burgdorferi* DNA has been detected in CSF from a limited number of these patients [163].

The serological tests used most commonly to diagnose *B. burgdorferi* infection include enzyme-linked immunosorbent assay, indirect immunofluorescence assay and Western blotting [164]. In

most cases, serological findings are dependent on the duration of the disease and clinical manifestations. In the early stages, <50% of patients have detectable antibodies, predominantly IgM. In the late stages, seropositivity rises to 70–90%, with a shift from IgM to IgG antibodies. An antibody response to *B. burgdorferi* analysed by enzyme-linked immunosorbent assay and Western blotting, and interpreted according to the criteria of the Centers for Disease Control, is the current standard for diagnosis of Lyme disease [122]. In Europe, three different strains of *B. burgdorferi* contribute to the disease, in contrast to one strain in the USA. No single set of criteria for the interpretation of immunoblots results in a high level of sensitivity and specificity in all European countries [165], and antibiotic therapy may prevent an increase in specific antibodies. However, seroconversion may still occur after antibiotic therapy. Thus, if antibody titres are negative in early Lyme borreliosis, repetition of the tests has been recommended after *c.* 4 weeks [164]. In chronic disease, IgG antibody titres are usually high, and may remain so for several years, even after successful treatment. Rarely, patients with chronic disease remain seronegative, showing only a cell-mediated immune response to *B. burgdorferi* [166].

One possible cause of seronegativity is the formation of immune complexes by antigen-specific antibodies [167]. The great variability in serological test results between laboratories may be associated with differences in the test procedures used, different diagnostic cut-off values for the discrimination of positive or negative values, and different antigen preparations or strains of *B. burgdorferi* used to prepare the test. Western blotting has been recommended as a confirmatory test [168], but interpretation is often difficult because specific and cross-reactive bands can often appear close together. A promising approach to standardise Western blots is the use of a defined panel of recombinant antigens [169].

TREATMENT OF LYME DISEASE

Most patients treated for Lyme borreliosis have an excellent prognosis, although some patients treated for erythema migrans in recent series continue to have a variety of complaints after antibiotic therapy. One recent study in New England, USA, found that most Lyme borreliosis patients who were not feeling well 3 months after treatment had laboratory evidence of coinfection with *Babesia* spp. [170]. Patients with carditis and neurological disorders also tend to do well after treatment, although some adult patients have residual neurological deficits such as mild seventh cranial nerve palsy after treatment [171]. Oral doxycycline or intravenous ceftriaxone are usually effective in the treatment of Lyme arthritis, in combination with non-steroidal anti-inflammatory drugs [172]. If arthritis persists despite the completion of two courses of antibiotic therapy, intra-articular steroids, disease-modifying antirheumatic drugs or arthroscopic synovectomy may be introduced. After appropriate treatment of Lyme disease, a small percentage of adult patients continue to report subjective symptoms such as musculo-skeletal pain, neurocognitive difficulties, or fatigue that may last for years. This disabling syndrome has been termed post-Lyme disease syndrome. Clinically, it resembles chronic fatigue syndrome or fibromyalgia [173]. It occurs more frequently in adult patients with symptoms suggestive of early dissemination of the spirochaete into the nervous system, and particularly if treatment was delayed [174].

Treatment of Lyme disease in children is comparable to recommendations established for adult disease [175]. Doxycycline, however, should not be used in the treatment of children aged <10 years. Early manifestations (except cardiac or neurological involvement) are treated with oral amoxycillin (50 mg/kg/day) or oral doxycycline (100 mg/day in two doses) for 14–21 days. Meningitis, meningoradiculitis, cardiac manifestations, Lyme arthritis and chronic treatment-resistant arthritis are treated with intravenous ceftriaxone 50–75 mg/kg/day once-daily for 14 days. Alternatively, intravenous cefotaxime (150–200 mg/kg/day) for 14 days, oral amoxycillin (50 mg/kg/day) or oral doxycycline (100–200 mg/day in two doses) for 30 days may be used [77].

PREVENTION OF LYME DISEASE

Avoid tick bites

Hikers can reduce exposure to ticks by walking wide trails. Preferred dress is light-coloured clothing (to make recognition and removal of the ticks easier), with long sleeves that are tight at the wrists and long trousers that are tucked into

light-coloured socks. A hat should be worn in densely wooded areas [176]. Habitats that are heavily infested with ticks, such as wooded areas, should be avoided if possible.

Tick and insect repellents that contain n,n-dimethylmetatoluamide applied to the skin provide additional protection, but require reapplication every 1–2 h for maximum effectiveness. Neurological complications in children from either frequent or excessive application of n, n-dimethylmetatoluamide-containing repellents have been reported. However, the health risk is low when these products are used according to the instructions [177]. n,n-Dimethylmetatoluamide should be applied sparingly only to exposed skin, and not to a child's face.

Permethrin (synthetic pyrethroid) is an insecticide derived from the chrysanthemum family of plants. It is used as a spray only on cloth, and it is deactivated on the skin. Once it is sprayed on to clothing, it becomes odourless. The effect of one single application can last for several weeks. Once it is applied, most ticks which come into contact with it will curl up, fall off, and eventually die.

Most studies show that transmission of *B. burgdorferi* from infected ticks usually requires a prolonged duration of attachment (12–48 h) to the host [178]. Therefore, attached ticks should be removed quickly with the help of medium-tipped tweezers as close to the skin as possible. If remnants of bite apparatus remain embedded in the skin, they should be left there. These are usually extruded parts, and additional attempts to remove them often result in unnecessary damage to tissues, which may increase the risk of local bacterial infection [136].

Eradication of ticks

In endemic residential areas, clearing bushes and trees, removing leaf litter and removing wood piles has been suggested. Pesticides have been used to suppress the tick population in residential areas. Erecting fences to exclude deer and ensuring that pets are tick-free may also reduce exposure to ticks [177].

Vaccines

Vaccines for Lyme disease that use recombinant OspA (rOspA) as an antigen have been produced by two manufacturers and field-tested for safety and efficacy in humans [136]. LYMErix (SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA) contains 30 mg of purified rOspA lipidated protein combined with 0.5 mg of aluminium adjuvant. This is the only licensed Lyme disease vaccine at this time, but marketing has been discontinued because of limited demand. Imulyme, a vaccine produced by Pasteur Merieux Connaught (Swiftwater, PA, USA), which contains 30 mg of purified rOspA lipidated protein without adjuvant, has not been propagated further.

rOspA vaccines have a unique mode of action. OspA is expressed by *B. burgdorferi* organisms that reside in the mid-gut of dormant ticks. *B. burgdorferi* subsequently down-regulates the expression in response to a blood meal and in preparation for skin invasion. Thus, ticks must become engorged with blood before they can transmit the organism. Patients who have not been exposed previously to *B. burgdorferi* have little antibody response to OspA (at least in the early stages of infection). When an immunised host is bitten by a tick infected with *B. burgdorferi*, protective OspA antibodies of the host are ingested by the tick. These antibodies then destroy *B. burgdorferi* in the gut of the tick and thus prevent transmission to the host.

In a large clinical trial, Steere *et al.* [179] found that the efficacy of LYMErix in preventing infection was 83% in the first year and 100% in the second year. In another large trial, Sigal *et al.* [180] found that the efficacy of the Imulyme vaccine in preventing symptomatic Lyme disease after the third injection was 92%. Some of the differences in the efficacy of the vaccines between the two studies may be explained by differences in methods of surveillance. The rOspA vaccines used in both clinical studies appeared to be safe. The vaccine-related side effects reported most frequently were pain, redness and swelling at the sites of injection [136]. These effects were usually mild and limited. The rOspA vaccine does not protect all recipients from infection with *B. burgdorferi* and provides no protection against other tick-borne diseases [136]. Therefore, vaccinated persons should also continue to take personal protective measures against tick bites.

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Plaintiff's Deposition Exhibit 12

From:	Accommodations Coordinator
Sent:	Tuesday, November 30, 2021 3:10 PM
To:	Tarquinio, Sally W.
Cc:	Accommodations Coordinator
Subject:	Vax Medical Accommodation Request - Tarquinio (Sally)--Determination
Attachments:	DENIAL - Vax Medical Accommodation - Tarquinio (Sally) ssmcg.pdf

Good day, Sally,

Attached, please find the above-referenced determination.

Accommodations Coordinator

JOHNS HOPKINS
APPLIED PHYSICS LABORATORY

11100 Johns Hopkins Road
Laurel, Maryland 20723-6099
240-228-5000 • www.jhuapl.edu

November 30, 2021

To: Sally W. Tarquinio
Systems Engineer, Radio Frequency Systems Engineering Section (A2B-005)
Sensor Systems Engineering Group (A2B)
Air and Missile Defense Sector (AMDS)

From: Shawn S. McGruder Shawn S. McGruder Digitally signed by Shawn S. McGruder Date: 2021.11.30 15:06:16 -05'00'
Principal Accommodations Coordinator
Legal and Commercialization Branch (CBO)
Central Laboratory (CL)

Subject: Medical Exemption Request from COVID Vaccination—Denied

On September 14, 2021, subject to and consistent with a Presidential executive order and other applicable laws, APL announced that every staff member, including (but not limited to) full-time, part-time, temporary-on-call, new hires, interns, and remote workers, must submit proof of at least the first dose of a COVID-19 vaccine by October 15, 2021, and verification of any second vaccine dose by December 1, 2021. Subject to applicable laws, the only exceptions to this policy are for those who have an approved reasonable accommodation from the Accommodations Coordinator for medical or religious reasons.

On October 1, 2021, you submitted a Request for Medical Accommodation, seeking exemption from APL's COVID vaccination requirement. On October 11, 2021, you submitted nine-year-old medical documentation that you believe supports your request to be excused from receiving the COVID-19 vaccine and a brief one-line note from your provider dated September 27, 2021, that stated "chronic Lyme Disease + Lyme Induced Immune Dysregulation," in response to the form's request to "[p]lease provide this information in a separate narrative that describes the reason in detail" as to why the patient should not be immunized for COVID. On October 18, 2021, an Accommodations Coordinator asked for more current and explanatory documentation as to why the COVID-19 vaccine is contraindicated for your diagnosis of Lyme disease. We have not received any updated medical documentation from your provider that more fully explains why the vaccination is medically contraindicated for you. Additionally, you never submitted a signed request for medical records, which would have allowed APL's Medical Officer to follow-up directly with your provider to secure further clarification. On November 22, 2021, you met with an Accommodations Coordinator who reviewed and verified your request.

After considering all of the materials you submitted and consulting with all available resources, we have determined that your request for exemption from APL's COVID vaccination requirement is insufficiently supported. Lyme disease is not a medical contraindication to receiving the COVID-19 vaccine, according to the CDC and medical literature. Moreover, your provider has failed to explain specifically why your Lyme disease diagnosis would preclude you from being vaccinated.

Therefore, APL's Accommodations Coordinator has determined that your medical accommodation request is **DENIED** at this time. Without disclosing the confidential medical details that form the basis for our determination, your leadership will stand advised of this outcome.

While we recognize that this is not the outcome you had requested, we hope you will decide to stay on staff with APL. To remain in our employ, please submit, via encrypted email to COVID19@jhuapl.edu, proof of 1) at least a first dose of the vaccination by the close of business on Tuesday, December 7, 2021, 5:00 p.m.; and 2) as applicable, within 6 weeks of the first dose, verification of the second shot of a two-dose vaccination sequence. If you choose not to do so, you will be separated from employment with APL.

Should you wish to submit additional information for APL to consider in assessing a medical necessity for your exemption from APL's vaccination requirement, please provide that information to the Accommodations Coordinator.

Thank you for your cooperation and patience with the process.

Plaintiff's Deposition Exhibit 15

From: Accommodations Coordinator
Sent: Monday, December 6, 2021 12:33 PM
To: Tarquinio, Sally W.; Accommodations Coordinator
Cc: Billups, Amy J.; Uy, Geoffrey S.; Dockery, G. D. (Dan)
Subject: RE: Additional information regarding my Accommodation Request

Dear Sally,

This morning, we carefully reviewed and consulted with APL's Medical Officer on the additional medical information you supplied late last week. Because the basis of your determination does not turn on whether you have established the underlying diagnosis below but, rather, whether you have established a condition that is contraindicated for receiving the COVID-19 vaccination according to CDC guidelines, the additional information does not change the determination you received last week.

Please work with your Group Supervisor and Strategic Partner to understand the options available to you. Thank you for the opportunity to review.

Accommodations Coordinator

From: Tarquinio, Sally W. <Sally.Tarquinio@jhuapl.edu>
Sent: Friday, December 3, 2021 4:17 PM
To: Accommodations Coordinator <Accommodations-Coordinator@jhuapl.edu>
Cc: Billups, Amy J. <Amy.Billups@jhuapl.edu>; Uy, Geoffrey S. <Geoffrey.Uy@jhuapl.edu>; Dockery, G. D. (Dan) <Dan.Dockery@jhuapl.edu>
Subject: FW: Additional information regarding my Accommodation Request

Correction: My CD57 count in 2012 was 29 (not 22).

From: Tarquinio, Sally W.
Sent: Friday, December 3, 2021 3:53 PM
To: Accommodations Coordinator <Accommodations-Coordinator@jhuapl.edu>
Cc: Billups, Amy J. <Amy.Billups@jhuapl.edu>; Uy, Geoffrey S. <Geoffrey.Uy@jhuapl.edu>; Dockery, G. D. (Dan) <Dan.Dockery@jhuapl.edu>
Subject: Additional information regarding my Accommodation Request

Attached is the CD57 results of my recent LabCorp blood test.

Most standard blood tests that are covered by health insurance are very insensitive. The CD57 blood test is used by Lyme-literate doctors to screen for the possibility

Plaintiff's Deposition Exhibit 16

JOHNS HOPKINS
APPLIED PHYSICS LABORATORY

11100 Johns Hopkins Road
Laurel, Maryland 20723-6099
240-228-5000 • www.jhuapl.edu

December 7, 2021

Sally W. Tarquinio
524 Saltworks Court
Annapolis, MD 21401

Dear Sally,

On September 14, 2021, the Laboratory announced a policy that followed the guidance set by President Biden and the U.S. government's initiative to combat the spread of COVID-19. Per that policy, all staff were required to provide proof of at least the first dose of an approved COVID-19 vaccine by October 15, 2021, or alternatively, to submit a request for a medical or religious accommodation to APL's Accommodations Coordinator.

You submitted a request for an accommodation, which was reviewed and considered, but ultimately denied. You were given one week to comply with the policy, but failed to provide proof of vaccination. As a result of this violation of policy, your employment with the Laboratory is terminated as of the date of this letter. If you are retiree eligible, your termination will be processed as a retirement and you will receive information related to the retirement process from the APL Staff Benefits Office.

As a result of this action, for two years from your date of termination, you are not eligible for subsequent employment by the Laboratory or for work at the Laboratory as a leased worker, independent consultant, subcontractor or in any other capacity. Also, during this period, you are not eligible to participate in any of the Laboratory's events, clubs or Affinity groups. Your final pay will be reduced to reflect any vacation or illness leave that you have previously been paid for, or recently taken, but have not yet earned.

We wish you well in your future endeavors.

Amy Billups
Group Supervisor, A2B

cc:
A. Billups
Office of Advising and Counseling
Talent Information Services

Plaintiff 001

Plaintiff's Deposition Exhibit 21

EXHIBIT 04

From: Semmel, Ralph D.
To: +Restricted APL All Staff List
Subject: Change to Vaccination Policy
Date: Tuesday, September 14, 2021 1:54:29 PM

Dear Colleagues,

Last Thursday, President Biden announced a new national strategy to combat the spread of COVID-19, which included an executive order that mandates workplace safety protocols for all federal contractors. With this order, the President clearly stated his intent that all employees of federal contractors be fully vaccinated. Consistent with this direction, our previously announced option of testing in lieu of vaccination is no longer appropriate.

Given the new guidance, every staff member must submit proof of at least the first dose of a vaccine by 15 October via the forthcoming Vaccination Verification System (VVS), which will be discussed in a follow-on message. All those who begin a two-dose vaccination sequence must also submit verification of the second shot by 1 December via the VVS. This policy will apply as a condition of employment to all staff, including but not limited to full-time, part-time, temporary-on-call, new hires, interns, and remote workers. The only exceptions to the policy are for those who have an approved reasonable accommodation from the Americans with Disabilities Act Coordinator for medical or religious reasons. Those seeking such an accommodation must submit their requests to the ADA Coordinator by 1 October.

Since we are one month away from this vaccine mandate, we are eliminating the "test or vaccination" policy that was to be implemented on 1 October and will instead focus on preparing for the new 15 October vaccination milestone.

I know that some of you will not be pleased with this change, but our country has reached a fundamentally different point in its nationally directed efforts to end the pandemic. It is now time for us at APL to move on and to refocus our efforts on the increasingly critical challenges facing our nation. For those who feel that this condition of employment is overly burdensome, I hope that this next month provides you with an opportunity to step back and reconsider how critical comprehensive vaccination is for the Lab and our nation. If you ultimately feel that you cannot abide by the change, and I truly hope that is not the case, then the coming month should provide you with sufficient time to work through transition plans.

On a personal note, having survived a bad case of long-haul COVID, I know how merciless this disease can be. Although I have natural immunity, which several of you have asked about as an alternative to vaccination, I got vaccinated because I knew it more effectively protected me and, more importantly, those around me.

It has indeed been a challenging eighteen months, and we are all looking forward to brighter

EXHIBIT 04

days. Vaccinations have proven to be essential in our efforts to build that more hopeful future. I encourage those of you who have not yet been vaccinated to join me, the vast majority of our APL colleagues, and the billions of others around the world who have been vaccinated, both to protect everyone in our communities and to enable us to more effectively serve and strengthen our nation.

Ralph